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Bioassay and Chemical Analysis for Hazardous Materials in Residual Oils. Volume 1. Narrative

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Bioassay and Chemical Analysis for Hazardous Materials in Residual Oils

Volume 1. Narrative

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a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pollution	Pollution Control	13B
Residual Oils	Stationary Sources	11H
Toxicity	Hazardous Material	06T
Chemical Analysis		07D
Bioassay		06A
Mutagens		06E
Radioactive Isotopes		18B

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ABSTRACT

The chemical composition of residual fuel oils and their column chromatography pre-fractions have been examined and mutagenic screening of them performed. A degree of correlation between composition and mutagenicity has been achieved. Radionuclide data has been obtained. A novel sample processing technique of solvent partitioning followed by column chromatography has been developed. It concentrates mutagenic factors into fractions which still retain their chemical class distinctions while eliminating sample components that are not of environmental concern. An as received residual fuel oil is either non-mutagenic or only weakly mutagenic. In this respect residual oils would not be considered environmental hazards. A residual oil is capable of being fractionated so that fractions will be mutagenic. Some fractions from some oil samples can be strongly mutagenic. Whether physical or chemical processes in usage or in the environment could act to concentrate or create mutagenicity is beyond the scope of this contract.

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NOMENCLATURE

CORPORATE IDENTITIES

W	Westinghouse Electric Corporation R&D Center, prime contractor of this project
SRI	Stanford Research Institute International, Inc., subcontractor performing Ames bioassay test
LBI	Litton Bionetics, Inc., subcontractor performing the yeast and *E. coli* bioassay test
CEP	Controls for Environmental Pollution, subcontractor performing radionuclide assay

GENERAL ABREVIATIONS

cm	centimeter	mm	millimeter
g	gram	ppm	parts per million
g	gravity	μg	microgram
mg	milligram	μl	microliter
ml	milliliter		

CHEMICAL SYMBOLISM

Chemical Elements

Al	Aluminum	Cl	Chlorine
Ag	Silver	Co	Cobalt
As	Arsenic	Cr	Chromium
B	Boron	Cu	Copper
Ba	Barium	F	Fluorine
Be	Beryllium	Fe	Iron
Bi	Bismuth	Ge	Germanium
Br	Bromine	H	Hydrogen
C	Carbon	Mg	Magnesium
Ca	Calcium	Mn	Manganese
Cd	Cadmium	Mo	Molybdenum

Chemical Elements (Cont)

N	Nitrogen	Se	Selenium
Ni	Nickel	Si	Silicon
O	Oxygen	Sn	Tin
P	Phosphorus	Sr	Strontium
Pb	Lead	Ti	Titanium
Re	Rhenium	V	Vanadium
S	Sulfur	Zn	Zinc
Sb	Antimony	Zr	Zirconium

Chemical Compounds

$AgNO_3$	Silver Nitrate	KCl	Potassium Chloride
CH_2Cl_2	Methylene Chloride	K_2HPO_4	Potassium Hydrogen Phosphate
CS_2	Carbon Disulfide		
FeS	Iron Sulfide	KH_2PO_4	Potassium Dihydrogen Phosphate
Ga_2O_3	Gallium Oxide	$MgCl_2$	Magnesium Chloride
HCl	Hydrochloric Acid	Na_2CO_3	Sodium Carbonate
HNO_3	Nitric Acid	$NaOH$	Sodium Hydroxide
H_2O	Water	Na_2SO_4	Sodium Sulfate
H_2SO_4	Sulfuric Acid	$(NH_4)_2SO_4$	Ammonium Sulfite

BIOASSAY TERMINOLOGY

D4	*Saccharomyces cerevisiae*, a yeast
DMSO	Dimethylsulfoxide (a partitioning solvent)
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*, a bacteria
NADP	B-Nicotinamide Adenine Dinucleotide Phsophate, Sodium Salt
plt	plate
$polA^+$	*E. coli*: strain W3110
$polA1^-$	*E. coli*: strain p3478
R	R ratio, the number of revertant colonies counted on the test plate over the number of revertant colonies counted on the control plates
S9	rat liver homogenate

BIOASSAY TERMINOLOGY (Cont)

TPN	Triphosphopyridine nucleotide [a synonym for NADP]
WP_2uvrA^-	A strain of *E. coli*

ANALYTICAL CHEMISTRY TERMINOLOGY

DIP	Direct Insertion Probe (for mass spectroscopy)
GC	Gas chromatography
GC/MS	Combined Gas Chromatograph-Mass Spectroscopy)
IR	Infrared
MS	Mass Spectroscopy
OES	Optical Emission Spectroscopy
SCOT	Support Coated Open Tubular [Gas Chromatography Columns]
UV	Ultraviolet

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We want to express our appreciation for and acknowledge the contribution of S.L. Rakes who served as the EPA project officer. We wish to show gratitude to G. L. Carlson, Manager, Westinghouse R&D Analytical Chemistry Department for his overall guidance.

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J. S. Rudolph	X-Ray Fluorescence Analyses
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M. L. Theodore	Halide Analyses
A. L. Wolfe	Residual Oil Sample Procurement
C. R. Wolfe	Contract Procurement and Initial Work Phases

Special thanks are due to J. A. Burger who first observed that there could be a problem in using automated colony counting in "reading" bioassay plates.

EXECUTIVE SUMMARY

I. What Was Done?

1. Bioassay of residual fuel oils.
2. Bioassay of fractions from residual fuel oils.
3. Organic analyses of fractions from residual fuel oil.
4. Inorganic analyses of residual fuel oils and fractions.

II. Why Was It Done?

1. To determine the potential environmental and public health hazards of residual fuel oils.
2. To determine the potential hazards of materials that could be concentrated from an oil in use or in the environment.
3. To pre-separate an oil to a point where hopes of isolation/characterization of hazardous organic species would be realistic.
4. To determine if there are any potentially hazardous inorganic materials in a residual fuel oil.

III. What Was Learned?

1. Chemical characterization is severely limited by low sample volatility.
2. Bioassay and chemical characterization of a residual fuel oil should procede in sequence rather than in parallel.
3. An as-received residual fuel oil may or may not be mutagenic.
4. All oils tested can be fractionated so that a particular fraction will be mutagenic.

5. Dimethyl sulfoxide partitioning may be a simple way for benefacting a hazardous residual fuel oil.

6. The major portion of the mutagenicity of a residual fuel oil does not reside in "classic" polycyclic aromatic hydrocarbons.

7. Model compounds and analytical reference data to aid in the identification of the type(s) of mutagens in a residual fuel oil are sparse at best.

8. The chemical class(es) containing the majority of the mutagenic factors can be basically aromatic or aliphatic in nature. With aliphatic compounds the degree of mutagenicity is probably less and may in fact be non-existent.

9. Since the major portion of the mutagenicity of a residual fuel oil involves "non-classical" compounds, correlation of mutagenicity (Ames test) with carcinogenicity (animal tests) will have little literature support.

IV. What Does It Mean?

1. Each as-received residual fuel oil must be individually assayed for bio-hazards.

2. Each residual oil tested as fractions contains mutagenic factors which could conceivably be concentrated in use or in the environment.

3. Given proper financial climate, it is conceivable that hazardous materials could be removed from a residual fuel oil prior to usage.

4. Identification of the mutagenic chemical types in a residual fuel oil would be a significant addition to the literature on chemical bio-hazards.

V. What Remains To Be Done?

1. Verification of the mutagenic potency of (our) fraction #3 of a residual fuel oil on a statistically valid basis.
2. Correlation between the dimethyl sulfoxide-soluble portion of a residual fuel oil and (our) fraction #3 of the oil in terms of a bio-hazard.
3. Continuation of the characterization of the mutagenic factors in (our) fraction #3 of a residual fuel oil, to include oils other than oil #16.
4. Characterization of the mutagenic factors in (our) column fractions from the dimethylsulfoxide soluble portions of residual oils.
5. Development of a sample separation technique, applicable to residual fuel oils, which will tend to separate on the basis of chemical compounds rather than on the basis of chemical classes.
6. Determination of the best practical technology (BPT) for reducing the bio-hazards of residual fuel oils for usage.
7. Correlation of mutagenicity of a residual fuel oil with carcinogenicity. This should be done through selected testing by the next screening tier - using mammalian cell lines, specifically the Syrian Hamster Embryo (SHE) cell transformation test.

SECTION 1

INTRODUCTION

The purpose of this contract has been stated succinctly by the project officer at one of the contract review meetings. The purpose is to "examine residual oils in detail; provide expanded, updated evaluation and assessment to allow estimation of environmental impact."

Residual fuel oils have been considered as energy source for Chemically Active Fluidized Bed (CAFB) process, designed to produce a low-sulfur, low-Btu fuel gas suitable for power-plant utilization in a conventional steam generator. This project was designed to assess the presence of potential bio-hazardous substances in residual fuel oils.

In spite of the length of time residual fuel oil has been available as a product, there has been little definitive study of what would be typical compositions for such materials (or whether even a composition can be "typical"). There has been less effort to assay this product for bio-hazard, and there has been no real effort to associate possible bio-hazard with composition. The very nature of refinery processes leads to residual oils which will contain polycyclic materials; be expected also to have nitrogen, oxygen, sulfur heteroatoms; and be appended with diverse functional groups. All these types of materials would be suspect as potential carcinogens.

This contract is for performing bioassay and chemical analyses on residual fuel oils. The bioassay work would have been simplified if the contract wording had been followed in performing bioassays only on as received oils. However, Westinghouse (in the spirit of the contract and with project officer approval) decided to apply the bioassay to particular oil fractions in an effort to more deeply probe potential carcinogenicity of this type of material. It was determined early on that, in terms of the 26 as received materials so evaluated, mutagenicity (i.e., the accepted measure of potential carcinogenicity) was not a serious concern with an as-received residual oil. There was no requirement nor was work done to evaluate what usage or environmental exposure would do to potential carcinogenicity of a residual oil.

The charter on analytical analyses was less sharply defined in that any and all information on sample composition was desired. Effort in this area therefore consisted of very close interface with the project officer in terms of what has been found, what does it mean and what is the best path to take from here. The major difficulties associated with these chemical analyses were sample complexity, low sample volatility, and lack of reference data.

The work begun under this contract is complete according to the work statement. Specific questions arising from this project need future answers, especially the discovered novel means of further resolving the difficulty of sample processing for environmental impact analyses.

SECTION 2

SUMMARY AND CONCLUSIONS

The data bank for writing this report arises in most part from the following:

41	Residual Fuel Oil Samples Received
26	As Received samples Bioassayed
32	As Received Samples Pre-Fractionated by Column Chromatography into Seven Fractions each
23	Samples Examined by Combined Gas Chromatography/Mass Spectroscopy/Data System as Seven Fractions Each
6	Samples Bioassayed as Seven Fractions Each
1	Sample Partitioned into Dimethylsulfoxide Solubles and Insolubles and then Pre-Fractionated by Column Chromatography into Seven Fractions Each
1	Sample, Partitioned and Pre-Fractionated as Above, Examined by Bioassay and Gas Chromatography/Mass Spectroscopy/Data System.

Direct insertion mass spectrometry, ultraviolet fluorescence, infra-red analyses and elemental composition studies have been performed as appropriate.

As received residual fuel oils can be either mutagenic or non-mutagenic. The observed positive mutagenicity would be termed very weak. Residual fuel oils can be fractionated by column chromatography so that, within the scope of this effort, a particular fraction is consistently mutagenic, even with an oil that is not mutagenic as received. As with as received oils, this particular fraction will show varying degrees of mutagenicity. The most powerful tool for isolating mutagenicity of a residual oil, as discovered here, is by dimethylsulfoxide partitioning of the as received sample followed by column chromatography fractionation of the dimethylsulfoxide soluble portion. In the single test run, each of the seven fractions obtained was mutagenic.

Four major factors affect the analyses for organic compounds in residual fuel oil:

1. The sheer complexity of the sample in terms of the hundreds and hundreds of compounds it contains.
2. The inconsistent source, treatment, and post-treatment blending from sample to sample.
3. The essentially non-volatile nature of residual fuel oils - this procludes complete sample analyses by the most powerful analytical tool, combined gas chromatography/mass spectroscopy/data system.
4. The lack of reference data to tie the sample components to known materials.

The organic analyses obtained in this program suggest:

1. The most mutagenic factors of a residual fuel oil are associated, in class with carbazole-type compounds.
2. The second most mutagenic factors of a residual fuel oil are associated, in class, with polycyclic ketones such as anthraquinone and benzanthrone. It is possible that there are concentration related toxic efects that prevent these factors from being bioassayed as the most mutagenic factors.
3. Major mutagenic factors in residual fuel oils are not associated with "classical" ones such as polycyclic aromatics (e.g. αBenzopyrene).

The elemental composition of residual oils is elucidated to some extent herein. The nature of inorganic material occurrence is such that it does not appear these material can be solubilized as required for performance of bioassay testing.

Radionuclide assay results for 11 isotopes on 28 as received residual fuel oils are presented.

SECTION 3

RECOMMENDATIONS

Based on the work that has been performed, the following recommendations can be made:

1. There is little analytical reference data to connect materials of interest in residual fuel oils to known compounds. Therefore it seems logical that continued effort to identify mutagens in residual fuel oils will add to the known list of such bio-active materials. The work of this contract should be continued.
2. It appears that mutagenicity of residual fuel oils does not reside principally in "classical" polycyclic aromatic hydrocarbons. It is therefore possible that accepted "relationships" between the Ames test and potential carcinogenicity of residual oil components may not be reflected in the literature and may not completely or at all follow expected trends. Residual fuel oils should be examined by second tier bioassay such as syrian hamster embryo cells, chinese hamster ovary, sister chromatid exchange or mouse lymphoma system.
3. Any mutagenicity of an as received residual fuel oil as studied in this work has been very weak. Fractions from this oil as obtained particularly from dimethylsulfoxide partitioning/column chromatography can be very strong mutagens. A test plan should be formulated and carried out evaluating the potential of usage/environment concentrating these latter factors, and at the same time checking undesirable usage/environmental alterations which a residual fuel oil can undergo.

4. Further chemical identification of mutagenic species should be carried out. This would certainly include use of our dimethylsulfoxide partitioning/column chromatography technique. It would also include re-evaluation of the well known "separation by classes" technique of Fuson and Shriner which could now be effective since presumed obstructive and inconsequential matrix (i.e. dimethylsulfoxide insolubles) is removed from the sample. This effort should also include attempts to upgrade the column chromatography method.

5. The feasibility of developing dimethylsulfoxide partitioning and/or the phenomenon of ultraviolet fluorescence as a "field test" for mutagenicity should be explored.

6. Each of the above recommendations should be applied for chemical analyses and genetic toxicology examination of materials, product and waste, from other energy conversion processes such as coal gasification, coal liquefaction and shale oil extraction.

7. The collection of 41 residual fuel oil samples assembled here is unique and probably could never be duplicated. The 15 as received oils not so examined should be bioassayed by Ames test. Given the "individuality" of composition of such samples all 41 should be subjected to DMSO partitioning followed by column chromatography. The final fractions should be bioassayed and examined by organic chemical analysis.

8. Any work as above should be performed in serial, rather than parallel, fashion consisting of the sequence: sample separation, bioassay, chemical analysis; with results of a prior operation determining whether there will be a subsequent operation.

SECTION 4

ENVIRONMENTAL IMPACT

While a body of literature is being formed on the study of crude oil and the more important commonly known refined products, virtually no studies have been made on the potential toxicity and mutagenicity of residual fuel oils. A survey of the literature reviewed by *Chemical Abstracts* from 1967 to the present revealed no obvious references to information concerning the environmental impact of the release of residual oils into the biosphere.

The residual oils are rather complex and undefined mixtures of products from the thermal and catalytic cracking processes of crude petroleum refining. Since their use as an energy source has been rather limited, and the residual oils are not normally released in quantity to the environment, they have not been characterized as well as the emerging synfuels and the more widely used products of oil refinery outputs. It would appear that the only opportunity for residual oils to be released into the environment in any significant quantities would be in the case of an accidental spill during transportation to the ultimate use site.

Since petroleum contains a complex of aromatic and heterocyclic compounds, including the well known carcinogens, benzopyrene and benzanthracene; residual fuel oils may contain classes of chemicals which include known or suspected carcinogenic substances, such as polycyclic organic compounds. However, it was found by Epler, *et al*[1] that the total "mutagenic potential" of a synthetic oil was much higher than that of natural crude oil in the *Salmonella* histidine-reversion assay. The detection or the generation of mutagenic activity may well be a function of the chemical fractionation scheme utilized before the chemical components are tested for mutagenic properties.

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[1] Epler, J. L., *et al*. (1978). Integrated Chemical and Biological Testing of Synthetic Oils and Effluents-Part I Short-Term Mutagenicity Testing.

Indeed, residual oil compositions vary widely from oil to oil and from refinery batch to batch. For typical residual oils, sulfur concentrations range from 1% to 6% by weight; nitrogen content varies from 1,000 to 6,000 ppm by weight; and trace elements vary over even broader ranges. Vanadium and nickel are the two most important indicators of metal content in residual oils.

Preliminary engineering analysis results[1] indicated that, in all probability, only a few inorganic pollutant compounds, such as vanadium, nickel, and chromium compounds, will pose any significant hazard in residual oil process gaseous effluents when used in power generation or industrial steam generation applications. Most of the potentially hazardous pollutants will be emitted as components of the waste-water or sludge streams of residual fuel oil utilization systems. Tyndall, et al. (1978) state that "fundamental data needs to be developed in high-temperature and high-pressure operations involving polynuclear aromatic derivatives and the prevalence of H_2, H_2S, CO, CO_2, NH_3, COS, HCN, and H_2O in products of residual oil utilization."

While the possible consequences of biologic effects of the residual fuel oils should not be ignored; it appears that there is no definitive evidence to indict the residual oils as potentially significant toxicants or mutagens or constitute on environmental hazard of serious consequence.

This contract on residual oil hazards supports the general conclusions presented. The bioassay of the 26 oil residues showed no mutagenicity in the Saccharomyces cerevisiae D4 strain or E. coli WP_2 uvr A -

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[1] Tyndall, M. F., et al. (1978). Environmental Assessment for Residual Oil Utilization - 2nd. Ann. Report (done for EPA by Catalytic, Inc.) (on microfiche).

organism at 2500 μg concentration and in some tests at levels as high as 5000 μg per plate of test organisms. The Samonella/microsome mutagenicity test (Ames test) was performed on the same 26 as-received oil samples, solubilized in p-dioxane and at the same 2500 μg levels per plate. The SRI found only 11 (42%) of the samples positive for frameshift mutation. A retest by W of these 11 positive residual oils, resulted in only 5 (or 19% of 26) being positive for frameshift mutation. The response levels were weak as compared to known mutagens. Three of the most reactive samples, positive both in SRI and W tests, were examined for DNA repair damage using E. coli Pol A^+/Pol A1 - strain. No effect on DNA repair system was present from these samples.

The Ames test is a very sensitive mutagenicity screening test for most classes of chemical, especially the organic aliphatics and polycyclic aromatics. This test detects substances of mutagenic activity at nanogram levels. While this system supplies useful genetic and toxicological information, its major criticisms are the low level of cellular organization in the test organism (bacteria) as related to mammalian cells, and inability to include all mammalian or human biotransformation and pharmacokinetic parameters. Yeast has been selected to eliminate the first cellular organization objection. Yeast has an organized nucleus similar to higher cell-types, and cytoplasm with various organelles found in mammals. Preliminary studies indicate that it may not be as sensitive as bacteria in the Ames test, but on the other hand it is reported to detect activity not detectable by the Ames test bacteria.

There are no figures available on the percent of false positives or false negatives, which can be expected with the yeast assay similar to the often quoted 80-90% accuracy with the Ames test. One could expect from the results to date that the accuracy of the yeast test would approach that of the Ames test but would not exceed it. No single bioassay system will ever be 100% accurate, which is the reason why several screening tests must be used to assay the mutagenicity of a compound. As an indication of the amount of information which has been produced in reports concerned with S. cerevisiae and mutagenicity, there are 578 such reports in the files at the Environmental Information Center at Oak Ridge as of mid 1979.

The E. coli WP_2 uvrA- is a complementary test to the Ames bioassay. The background test information of this system is the least developed of the test systems used. When the three test results are combined however, a more complete picture of the potential genetic effect from a substance can be estimated. As the results of this project indicate, the probability of adverse genetic effects, or for that matter any genetic effect, from residual oils as tested, is very low. Two genetic, screening systems are negative and the most sensitive system - Ames test - produces a positive response in only 42% of samples with a liberal interpretation and 19% with retesting. The changes in mutagenicity encountered in this study indicate that the active agent(s), when carried in a solvent (p-dioxane), will change its biological activity with time, thus the "active" life of the material is limited. The fractionation of the samples, again tested at 2500 µg levels as maximum, gave relatively weak response, although the potential mutagenic agent is actually concentrated in these tests. In addition, the residual oils are not water soluble.

In summary, no serious genetic hazard is seen from residual oil, should they accidentally escape into the environment because:

- Very sensitive Ames test gives very weak frameshift response in 19% or maximum 42% of samples tested.
- Two other mutagenicity tests - D4 and E. coli WP_2 - are negative.
- No DNA repair damage produced.
- Mutagenic material is not water soluble.

A certain amount of cytotoxicity was observed with the solubilized material. Aquatic and Terrestrial Ecological Effect Tests (EPA 600/7-77-043) for Level 1 and 2 IERL-RTP Environmental Assessment and the RCRA proposed guidelines were not performed, not being part of the project work statement. From the general evaluation of the project results it is concluded that residual oils should not present an environmental hazard, unless disposed into the environment in large, concentrated volumes. This is unlikely, except accidentally, due to their high value as source of energy.

SECTION 5

EXPERIMENTAL TECHNIQUES

This section describes only those techniques that would be applied to any continuation or expansion of this type of work. Other techniques developed/used that for one reason or another would not be expected to find continuing use are described as appropriate within the discussion of Section 6.

SAMPLE PREPARATION

According to current best thought, a residual oil should be partitioned with DMSO and the resultant two fractions subjected to column chromatography. On any work to clarify the manner of inorganic element occurence, dioxane and toluene partitioning of an as received sample may be helpful.

DMSO Partitioning

Ten to fifteen grams of the residual fuel oil sample is weighed, by difference, into a 1 pint Waring Blender jar. DMSO (250 ml) is added and the contents blended for three 2 minute periods at high speed. The mixture is allowed to cool somewhat between blending periods. The mix is filtered thru 12 1/2 cm Blue Ribbon filter paper under gravity. In the experiment performed for this contract, 97% of the DMSO was recovered as filtrate. In the work of this laboratory, the DMSO insolubles were re-extracted with additional DMSO (250 ml). Only an additional 1.1% of solubles were obtained.

The DMSO filtrate is admixed with 500 ml of water to produce an emulsion. This is extracted with eight 100 ml portions of hexane. The hexane portions are filtered as above. Some sludge may form in the hexane and/or aqueous phase during this process. It appears to be mutugenic (as in the one sample partitioned) and should be collected. It can be washed with methanol on a filter and then dissolved thru with toluene.

Column Chromatography

The column material preparation and column operation conditions for the further separation of the above DMSO partition fractions are as follows:

1. Fifty grams of anhydrous Na_2SO_4 (such as Baker #3898) is dissolved in one liter of water.
2. The solution is hand-mixed with 1000 grams 60-100 mesh Florisil (such as Fisher #F-100).
3. The resultant mixture is heated to constant weight at 110°C. It is cooled in a dessicator and stored in well-capped jars.
4. A weighed sample of 4 grams residual oil is dispersed in 10 milliliters methylene chloride (Burdick and Jackson distilled in glass).
5. Twenty-five grams of the Na_2SO_4/Florisil preparation are admixed into the sample/solvent.
6. The sample preparation is dried at 110°C for 16 hours and stored in a desiccator until used.
7. Seventy-five grams of the prepared Na_2SO_4/Florisil material are placed in a glass cylinder containing a fritted glass disc (Corning #33950-XLC Extraction Thimble).
8. The sample/Florisil/Na_2SO_4 (25 grams) is placed on top of the above 75 grams of material.
9. The prepared glass cylinder is placed inside a glass funnel with truncated stem and containing a cone of Whatman Blue Ribbon filter paper.
10. The following solvent systems are passed through the column by gravity flow, collecting all of a given system in one container

(all solvents except ethanol are Burdick and Jackson distilled in glass. The ethanol is 200 proof as supplied by Publicker Industries).

a. 600 ml Hexane
b. 600 ml Hexane with 15% Toluene
c. 600 ml Methylene Chloride
d. 600 ml Diethyl Ether with 3% Ethanol
e. 500 ml Methanol
f. 600 ml Methylene Chloride with 3% Ethanol
g. 500 ml Dioxane with 3% Ethanol.

The system has to be a closed one upstream of the column, with dry air make-up, both to eliminate solvent loss through evaporation and prevent introduction of water through condensation.

The collected solvent systems are evaporated to "dryness" as aliquots into a 150 ml beaker; under dry nitrogen in a warm water bath. There are three rinsings of the eluant collection beaker using heat to work a vapor ring almost completely up the side of the beaker. Hexane is used with the first two fractions, methylene chloride with the remaining five. Final fraction drying is for no more than 15 minutes at 110°C.

Dioxane and Toluene Partitioning

Because of difficulties in filtering due to clogging it is believed the following procedure has to be followed rather closely. A weighed sample between 2.5 and 3 grams is used. One hundred milliliters of dioxane are used to transfer the sample to a 47 mm 5 micron teflon filter in a standard millipore funnel, as follows: 10 ml of solvent are placed in the funnel with the vacuum turned off. Thirty ml of solvent is then mixed with the sample, and a few ml of the mixture added to the funnel. The vacuum is turned on and the funnel allowed to drain completely. The process is repeated until all the sample has passed through. Then the remaining 70 ml of dioxane is used for flushing the beaker and funnel. The filter is washed with another 100 ml of dioxane, and dried at 100°C for one hour.

Separating the toluene-insoluble material from the above fraction presents another problem. Using the above procedure results in almost immediate, complete, clogging of the filter. Even a large excess of toluene in the funnel before addition of one or two ml of sample solution does not help. The difficulty is remedied by warming both the sample solution and the toluene to around 60°C. With this modification to the dioxane procedure, filtration and washing can be completed in less than one hour. Washing is with 20 ml portions of toluene, the number of which is three more than required to give a clear filtrate. (The same rule was applied in washing the dioxane insoluble material.) A total of about 500 ml of toluene is used for transfer and washing. After the final wash, the filter is dried at 115°C for one hour, cooled and weighed. This yields the dioxane insoluble-toluene insoluble portion of the residual oil. The dioxane insoluble-toluene soluble is obtained by evaporating the toluene filtrate under dry nitrogen as for the column fractions above.

BIOASSAY

The bioassay test procedures employed in this contract were in compliance with the generally accepted methods as reported in the literature by the originator of the respective tests. Individual, minor changes are frequently used by different laboratories. The bulk of the bioassay work was performed on subcontract, hence each subcontractor's methods and protocol are presented as submitted by them. When W performed bioassays, it followed, without procedural change, those of the subcontractors. The only change between W and SRI Ames test was that W used three plates per solvent control and each concentration, as opposed to one each by SRI.

Yeast D4 and E coli - Litton Bionetics, Inc.

Purpose

The purpose of this study was to evaluate the test material for genetic activity in a microbial assay with and without the addition of mammalian metabolic activation preparations.

Materials

Indicator Microorganisms

A description of strain verification is given in Standard Operating Procedure on page 24.

Escherichia coli WP_2uvrA^-

Saccharomyces cerevisiae D4

Activation System

Reaction mixture

Component	Final Concentration/ml
TPN (sodium salt)	4 μmol
Glucose-6-phosphate	5 μmol
Sodium phosphate (dibasic)	100 μmol
$MgCl_2$	8 μmol
KCl	33 μmol
Homogenate S9 fraction	0.1 ± 0.05 ml

S9 homogenate

A 9,000 x *g* supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill according to the procedure of Ames *et al*. (1975). S9 samples were coded by lot number and assayed for milligrams protein per milliliter and relative P448/P450 activity by methods described in LBI Technical Data on Rat Liver S9 Product.

Positive Control Chemicals

The chemicals used for positive controls in the nonactivation and activation assays are given in Table 1 of Section V. Results.

Solvent

p-Dioxane was used to prepare stock solutions of solid materials. All dilutions of test materials were made in p-dioxane. The solvent employed and its concentration are recorded in Table 1 of Section V. Results.

Experimental Design

Reverse Mutation with E. Coli WP_2uvrA^-

Approximately 10^8 cells from an overnight culture of the indicator strain are added to separate test tubes containing 2.0 ml molten agar supplemented with a trace of tryptophane. For nonactivation tests at least four dose levels of the test compound and 0.5 ml of phosphate buffer pH 7.4 are added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, at least four dose levels of the test chemical are added to the appropriate tubes with cells. Just prior to pouring an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) is added to each of the activation overlay tubes. The contents are mixed, poured over the surface of a minimal agar plate and allowed to solidify. The plates are incubated for 48 hours at 37°C and subsequently scored for the number of colonies growing on each plate. Positive and solvent controls are run with each assay.

Mitotic Gene Conversion with Yeast Strain D4

Approximately 10^7 cells of the indicator strain from a culture grown overnight are added to test tubes containing 2.0 ml molten agar (supplemented with tryptophane for the activation test). Four dose levels of the test compound, 0.5 ml of S9 mix (for the activation assay) or 0.5 ml phosphate buffer, pH 7.4 (for the nonactivation assay), are added to the content of the appropriate tubes with cells. The contents of the tubes are mixed and poured over the surface of minimal agar plates and allowed to solidify. The plates are incubated at 30°C for three to five days and then scored. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation are run with each assay.

Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants (or convertants for D4) per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

Evaluation Criteria

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 days.

Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over 2 or 3 log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

Dose-Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assay. However, for the E. coli strain WP_2uvrA^- and yeast strain D4 there is no known pro mutagen that can be used in the plate incorporation assay using the activation system. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

Evaluation Criteria for Plant Incorporation Assays Using E. coli Strain WP_2uvrA^- and Yeast Strain D4.

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value is considered to be mutagenic. For these strains, the dose-response increase should start at approximately the solvent control value.

Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data lose significance.

The preceding criteria are not absolute, and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

All evaluations and interpretation of the data presented in this report are based only on the demonstration, or lack, of mutagenic activity.

Standard Operating Procedure

All data will be entered in ink (no pencil).

All changes or corrections in entries will be made with a single line through the change, and an explanation for the change must be written.

All calculations (weights, dilutions, dose calculations, etc.) will be shown on data records.

All entries will be dated and initialed.

All laboratory operations will be written out in standard protocol manuals. These manuals will be present in each laboratory area.

Deviations from any established protocol will be described and justified.

Data will be stored in bound form (notebooks or binders). These bound data books will be reviewed by the appropriate Section Heads.

Chemicals submitted for testing will have date of receipt and initials of entering person.

Lot numbers for all reference mutagens, solvent, or other materials used in assays will be recorded.

Animal orders, receipts, and identification will be recorded and maintained such that each animal can be traced to the supplier and shipment. All animals on study will be properly identified.

A copy of the final report plus all raw data and support documents will be permanently stored in the archival system of Litton Bionetics, Inc.

Current curricula vitae and job descriptions will be maintained on all personnel involved in the study.

Salmonella strains will be routinely checked for the *his*, *uvrβ*, rfa, and pKM 101 phenotypes. Only appropriately screened stock cultures will be used in chemical evaluations.

References

Brusick, D. and Andrews, H.: Comparison of the genetic activity of dimethylnitrosamine, ethylmethane sulfonate, 2-acetylaminofluorene and ICR-170 in *Saccharomyces cerevisiae* strains D3, D4 and D5 using *in vitro* assays with and without metabolic activation. Mutation Res., 26:491-500, 1974.

Green, M. H. L. and Muriel, W. J.: Mutagen testing using Trp+ reversion in *Escherichia coli*. Mutation Res., 38:3-32, 1976.

Ames Test - Stanford Research International, Inc.

Salmonella typhimurium Strains TA1535, TA1537, TA1538, TA98, and TA100

The *Salmonella typhimurium* strains used at SRI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium petri plates containing a trace of histidine, only those cells that revert to histidine independence (*his*$^+$) are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The *his*$^+$ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar the mutation frequency is increased 2- to 100-fold.

We obtained our S. typhimurium strains from Dr. Bruce Ames of the University of California at Berkeley.[1-5] In addition to having mutations in the histidine operon, all the indicator strains have a mutation (rfa$^-$) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of vitamin biotin (bio$^-$) and in the repair of ultraviolet (uv)-induced DNA damage (uvrβ^-). The rfa$^-$ mutation makes the strains more permeable to many large aromatic molecules, thereby increasing the mutagenic effect of these molecules. The uvrβ^- mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to his$^+$ by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the resistance transfer factor plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens.[5] In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of the plasmid in the cells. The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens [e.g., ICR-191, benzo(a)pyrene, aflatoxin B_1, and 7,12-dimethylbenz(a)anthracene]. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. TA1537 is more sensitive than TA1538 to mutation by some acridines and benzanthracenes, but the difference is quantitative rather than qualitative. Strain TA98 is derived from TA1538 by the addition of the plasmid pKM101, which makes it more sensitive to some mutagenic agents.

All indicator strains are kept at 4°C on minimal medium plates, supplemented with a trace of biotin, and an excess of histidine. The plates with the plasmid-carrying strains contain in addition ampicillin

(25 µg/ml), to ensure stable maintenance of the plasmid pKM101. New stock culture plates are made every two months from single colony reisolates that were checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of the plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid, CM67). After stationary overnight growth, the cultures sre shaken for 3 to 4 hours to ensure optimal growth.

Aroclor 1254-Stimulated Metabolic Activation System

Some carcinogenic chemicals (e.g., of either the aromatic amino type or the polycyclic hydrocarbon type) are inactive unless they are metabolized to active forms. In animals and man, an enzyme system in the liver or other organs (e.g., lung or kidney) is capable of metabolizing a large number of these chemicals to carcinogens.[4,6-9] Some of these intermediate metabolites are very potent mutagens in the *S. typhimurium* test. Ames has described the liver metabolic activation system that we use.[6] In brief, adult male rats (250 to 300 g) are given a single 500-mg/kg intraperitoneal injection of a polychlorinated biphenyl, Aroclor 1254. This treatment enhances the synthesis of enzymes involved in the metabolic conversion of chemicals. Four days after the injection, the animals' food is removed but drinking water is provided *ad libitum*. On the fifth day, the rats are killed, and the liver homogenate is prepared as follows:

The livers are removed aseptically and placed in a preweighed sterile glass beaker. The organ weight is determined, and all subsequent operations are conducted in an ice bath. The livers are washed in an equal volume of cold, sterile 0.15 M KCL (1 ml/g of wet organ), minced with sterile surgical scissors in three volumes of 0.15 M KCl, and homogenized with a Potter-Elvehjem apparatus. The homogenate is centrifuged for 10 minutes at 9000 x *g*, and the supernatant, referred to as the S-9 fraction, is quickly frozen in dry ice and stored at -80°C.

The metabolic activation mixture for each experiment consists of, for 10 ml:

- 1.00 ml of S-9 fraction
- 0.20 ml of $MgCl_2$ (0.4 M) and CKl (1.65 M)
- 0.05 ml of glucose-6-phosphate (1 M)
- 0.40 ml of NADP (0.1 M)
- 5.00 ml of sodium phosphate (0.2 M, pH 7.4)
- 3.35 ml of H_2O.

Assays in Agar

To a sterile 13 x 100 mm test tube placed in a 43°C heating block, we add in the following order:

(1) 2.00 ml of 0.6% agar. 0.6% agar contains 0.05 mM histidine and 0.05 mM biotin.
(2) 0.05 ml of indicator organisms
(3) 0.50 ml of metabolic activation mixture (optional)
(4) 0.05 ml of a solution of the test chemical.

For negative controls, we use steps (1), (2), and (3) (optional) and 0.05 ml of the solvent used for the test chemical. Because the majority of organic compounds are not sufficiently water soluble--particularly at the higher concentrations--we routinely use dimethylsulfoxide (DMSO). Other solvents that are occasionally used are water, ethanol, or benzene. For positive controls, we test each culture by specific mutagens known to revert each strain using steps (1), (2), (3) (optional), and (4).

This mixture is stirred gently and then poured onto minimal agar plates. Minimal agar plates consist of, per liter, 15 g of agar, 50 g of glucose, 0.2 g of $MgSO_4 \cdot 7H_2O$, 2 g of citric acid monohydrate, 10 g of K_2HPO_4, and 3.5 g of $NaHNH_4PO_4 \cdot 4H_2O$. After the top agar has set, the plates are incubated at 37°C for 2 days. The number of $\underline{\text{his}}^+$ revertant colonies is counted and recorded.

References

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6. B. N. Ames, J. McCann, and E. Yamasaki. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res. 31, 347-364 (1975).
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DNA Repair - Westinghouse R&D Center

The DNA damage/repair test procedure adopted for routine use at the R&D Center is based on the Green and Muriel[1] methods. These methods were adopted with only very few minor modifications to comply with the established laboratory procedures of the Biology group. The modifications were tested, compared to the reported procedure, and found to have no adverse effect on the test results. The Green and Muriel procedure, with special emphasis on their bacterial media, was adapted to insure a smooth transition to the second generation, improved tester strains in preparation at their laboratory. The Green and Muriel procedure is a modification of test, originally designed by Slater, Anderson, and Rosenkranz in 1971[2].

The microorganism used in this test is Escherichia coli, strains W3110 and p3478, known as pol A^+ and pol $A1^-$ due to the induced mutations in their genetic code. The organisms were purchased from the American Tissue Culture Collection (ATCC), Rockville, MD. The strain W3110 (ATCC No. 27325) is a mutant strain deficient in thymine, but with an intact polymerase system -pol A^+. In contrast, strain p3478 (ATCC No. 25947) is deficient in the polymerase enzyme -pol $A1^-$, and was derived from W3110. Both strains are easily maintained on nutrient agar at 4°C, which is the method for keeping the stock cultures of each strains between tests.

The test requires that the chemical test substance diffuses into the agar from the paper disc, so that contact between the organism and the substance is made. The concentration of the test material varies inversly with distance from the disc. Solids are dissolved in water or when insoluble, in a non-toxic solvent. The organic solvent of choice is dimethylsulfoxide (DMSO), although p-dioxane without stabilizers has

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1. Green, M. H. L. and W. J. Muriel 1976. Mutagen testing using trp^+ Reversion in Escherichia coli. Mutat. Res. 38: 3-32.
2. Slater, E. E., M. D. Anderson and H. S. Rosenkranz 1971. Rapid detection of mutagens and carcinogens. Cancer Res. 31: 970-973.

been found to be an acceptable substitute. The usual practice for solids or viscous liquids is to solubilize 1 gram of the test material in 10 ml of DMSO. Under certain experimental conditions the step 5 - paper disc application - is omitted and the test substance is applied directly onto the agar surface either as crystal, a drop of viscous material, or 25 µl quantity of the test chemical as received or in an appropriate solvent. This modification is occasionally necessary when undiluted material must be tested or the disc interferes with the diffusion process. This in no way changes the test analysis. The only difficulty is in measuring a less uniform zone of inhibition, if one occurs.

The eight step test procedure, is discussed step by step as follows:

Step 1 - Prepare Selective Agar and the Plates

For testing, a minimum of 30 plates of selective agar are prepared for each 5 test substances assayed. Six plates, 3 per strain, are prepared for each of the test agents. The selective agar is made with the five components listed below. The first four are autoclaved separately before combining. The fifth, L-trypophan is filter sterilized.

Component 1

Difco Bacto-agar	15 g
Distilled water	750 ml

Component 2 (Davis Mingioli salts)

$K_2H\ PO_4$	7 g
$K\ H_2\ PO_4$	2 g
$(NH_4)_2\ SO_4$	1 g
Trisodium citrate	0.25 g
Distilled water	250 ml

Component 3

Dextrose	4 g
Distilled water	20 ml

Component 4

Difco casamino acid	2.5 g
Distilled water	25 ml

Component 5

L-tryptophan is prepared at the rate of 100 mg in 100 ml sterile water. One ml of this solution is added to components 1-4. This equals to 1 μg/ml.

The 30 plates are coded for identification after final inspection for contaminants.

Step 2 - Prepare Tester Strain Cultures

Incubate @ 37°C, 17 hours

A liquid growth medium is prepared for culture maintenance and for cell growth to the log phase of 2×10^8 cells/ml for tests.

The constituents of the liquid growth medium are listed below.

Component 1

$K_2H\ PO_4$	2.8 g
$K\ H_2\ PO_4$	0.8 g
$(NH_4)_2\ SO_4$	0.4 g
Trisodium citrate	0.1 g
$Mg\ SO_4 \cdot 7H_2O$	40 mg
Distilled water	400 ml

Component 2

Dextrose	1.6 g
Distilled water	8 ml

Component 3

Difco casamino acids	1 g
Distilled water	10 ml

Component 4

L-tryptophan	4 mg
Distilled water	4 ml

A single colony of each strain is removed from the stock culture petri plate and inoculated into separate flasks containing 10 ml of growth medium. Sterile 125 ml Erlenmyer flasks are used as the culture vessel. This large a size for 10 ml of fluid provides a high surface to volume ratio for necessary aeration. The flasks are incubated with agitation at 37°C for 17 hours. These are referred to as overnight or 17 hour cultures. These cultures may be used immediately at the end of the 17 hours incubation period for step 3 or they may be stored at 4°C and used within a week.

Step 3 - Incubate @ 37°C, 2.5 Hours for Logarithmically Growing Cultures of 2 x 10^8 cells per ml

An aliquot of 0.1 ml of the overnight culture from step 2 is inoculated into 10 ml of fresh growth medium. The cultures are incubated for 2.5 hours at 37°C in the same manner as the overnight cultures. The 10 ml of 2.5 hour culture contains sufficient cells for 85 plates of each strain when 0.1 ml is applied to each plate. If more than 85 plates of each strain are required (i.e. to test more than 14 test substance concentrations) another set of 2.5 hour is prepared. The volume of 10 ml of growth medium per flask should not be exceeded, to insure uniform cell count for all experiments.

Step 4 - Spread Inoculum with Glass Rod (3 plates/test agent)

Pipette 0.1 ml pol A^+ or pol $A1^-$ on the top of the agar and spread evenly The density of the cells can influence results, therefore, care must be exercised in spreading the inoculum.

Step 5 - Add Sterile Discs to Top of Agar

Half inch sterile blank paper discs are added aseptically to the top of the agar containing the bacteria. The discs are gently pressed against the agar to give maximum contact of the paper to the agar surface. The disc serves to localize the test agent and thus permits even diffusion around the disc into the agar rather than in an irregular pattern. Measurements of the zone of inhibition are thus more easily made.

Step 6 - Add Test Agent to Disc

A volume of 10 to 25 µl of undiluted test agent is applied to the blank disc. Larger volumes of 50 or 100 µl may also be tested. The agent is dispensed using a centaur automatic pipette with sterile disposable plastic tips. Diluted test agents are used only when the nature of the agent requires dilution. Care must be exercised not to cause the agent to flow off the disc before it is absorbed into the agar. Plates used to test volatile agents are segregated for incubation.

Step 7 - Incubate Test Plates at 37°C for 48 hours

The inhibitory effect can be observed after 7 hours but results are more definite after 48 hours as recommended by Green and Muriel.

Step 8 - Measure Zones of Inhibition (mm)

The bacterial growth on the agar produces a cloudy appearance. This is referred to as a "lawn". Areas where growth is inhibited are transparent. A calibrated calipher is used to measure the average distance between the disc to the solid lawn (zone of inhibition). This measurement in millimeters is recorded for each plate. The zones on the three plates are averaged.

A positive effect is recorded if the average zone of inhibition in the polymase deficient strain, pol $A1^-$, is greater than that of the strain with the intact system, pol A^+.

Results are reported as the ratio of the average zone of inhibition with pol $A1^-$ to the average zone of inhibition with pol A^+ $\left(\frac{\text{avg. pol } A1^-}{\text{avg. pol } A^+}\right)$. Numbers greater than unity are considered to be positive and may indicate that DNA damage was induced by the test agent. Negative results may mean no effect of the agent or the agent failed to contact the DNA.

Solubilization

Prior to submitting samples to subcontractors for analyses, a preliminary solubilization, cytotoxicity, and mutagenicity tests were performed on the first residual oil sample received at Westinghouse. This was Shell bunker oil #6 marked RO#1 for sequential numbering of future bioassay samples.

Preliminary Tests - Water and DMSO

The two primary solvents used in bioassays with bacteria, yeasts, or tissue culture cells are water and dimethylsulfoxide (DMSO). Water solubility with agitation at room temperature for 60 hours with 10 grams of oil in 100 ml of deionized water was unsuccessful. The water remained clear, indicating no appreciable oil solubility. Dimethylsulfoxide (DMSO) on the other hand left little residue, the solution becoming dark brown in color during 60 hour agitated exposure. Since DMSO is essentially non-toxic and is recommended as Ames test solvent, this was originally accepted as the residual oil solvent for all bioassay tests.

The drop test for cytotoxicity of RO#1 was performed on Salmonella typhimurium TA1535, TA1537, TA1538, TA98, and TA100 with the water and DMSO extracts using a 10 µl drop per plate. No toxicity (zone of inhibition) and no increase in the revertants over controls was observed with water or DMSO extract.

To further check out for potential cell damage effects in a full scale study, a drop test using Pol A^+/PolA1$^-$ E. coli for DNA damage repair was also performed with a 10 µl water and DMSO extract. Water produced no zone of inhibition beyond the drop which had about a 99% cell kill, and measured 1.8 cm and 2.3 cm in diameter for A^+ and A1$^-$ respectively. DMSO had no zone of inhibition. Cell survival under the drop was impossible to observe due to the dark brown precipitate formed by the extract. From this it was concluded that water and DMSO contain some cytotoxic substances in quantities too small to interfere with bioassay tests with proper dose level exposures. No DNA repair damage was produced by RO#1.

With this information the Ames plate test was performed using all five strains and the following dose levels for both water and DMSO extracts: full strength, 1:9, 1:20, 1:50 and 1:100 dilutions. No mutagenicity was observed with the water survey with and without enzyme activation. The DMSO extract showed no mutagenic activity without activation. With S9 Mix activation no required two fold increase was observed, however, in TA1538 and TA98 at full strength a one and one-half increase over the control was demonstrated. This indicates that there may have been a mutagenic agent(s) in the DMSO extract, but at concentrations too low to elicit a positive, frameshift response.

For the purpose of standardizing the solubilization to meet not only the bioassay but also analytical chemistry needs, the following procedure was set up. One gram of smaple (shell #6) + 100 ml DMSO, 3 mm, in blender, insolubles discarded; solubles filtered into H_2O emulsion centrifuged (no separation) and extracted with petroleum either organic extract (35% of original 1 gm) into DMSO at about 1% w/v aqueous portion discarded, organic extract (12% of original 1 gm) into 1% DMSO w/v. The two DMSO fraction were then tested on TA1535, TA1538, TA98, and TA100 with and without enzyme activation. Based on the previous results, no dilutions were made. Instead the extract at 1% w/v concentration was added to the soft agar overlay at 10, 50, and 100 µl/plate or 100, 500, and 1000 µg/plate.

The organic extract was not mutagenic in the base pair substitution strains of TA1535 and TA100. In the frameshift tester organisms of TA1538, and TA98, showed mutagenic, does-related activity with and without metabolic activation, especially without activation. The results were as follows using R ratio values only.

	TA1538		TA98	
µg per Plate	No Activation	Metabolic Activation	No Activation	Metabolic Activation
100	0.9	1.5	2.0	3.0
500	2.2	2.7	3.4	2.9
1000	3.4	2.8	4.5	2.3

It should be noted that TA98 appears to be more sensitive than TA1538, and that the S9 Mix seems to neutralize the mutagenic effect of the agent(s).

To further characterize the various residual oil fractions and insure bioassay of active portions, a volatile portion of RO#1, collected under vacuum at moderately low temperatures, was bioassayed. The total sample weight was 19 micrograms dissolved in 2 ml of DMSO. This gave the following concentrations: 10 μl - 95 μg; 50 μg - 475 μg; 100 μg; 100 μg - 950 μg. The small sample amount necessitated a judicious selection of tester strains. Based on the previous response, frameshift mutation is the only type to be expected with this material. Therefore, TA1538 and TA98 were employed. The sample was tested with and without metabolic activation. At 95 μg level no mutagenic activity was observed. The two higher doses were too toxic, resulting in a very poor or absent background lawn or no growth. The volatile portion was judged to be not significant for routine mutagenicity testing.

Final Solvent-p-dioxane

The low oil residue solubility of approx. 47% in DMSO necessitated a further search for another, non-cytotoxic solvent. The selection was narrowed to p-dioxane. The solubility is 97 + % of shell #6 oil. Dr. Ames has reported that p-dioxane is not toxic to the bacterial tester strains up to 100 μl/plate. A solution was prepared, containing 50 mg shell #6 in 0.1 ml p-dioxane or 500 μg/1 μl.

The drop test was performed on TA1535, 1537, 1538, 98 and 100, using 10, 50, and 100 μl/plate of solution (5000, 25,000 and 50,000 μg respectively) and 100 μl of p-dioxane alone. The results did show cytotoxicity alone or with the oil residue, when 100 μl were applied; ten and 50 μl with residue appeared to be acceptable. Increased revertants were observed in all but TA100 uniformly through the plate as compared to the p-dioxane control. The p-dioxane was also tested in D4 for toxicity. None was detected at the maximum levels of 100 μl per plate or tube used.

ORGANIC ANALYSES

Gas Chromatography/Mass Spectroscopy

Residual oil samples are injected into the GC/MS system as 1 µl, 10% solute in toluene. The column used for most of our work was either, SCOT OV101 or SCOT OV225, both 50 ft. by 0.020 inch. The column conditions are:

Initial Temperature	100°C
Initial Time	3 minutes
Temperature Rate	5°C/minute
Final Temperature	225°C for 225; 250°C for 101
Helium Flow Rate	approx. 15 ml/min.

The GC/MS conditions are:

Run Time	60 minutes
Start, Stop Masses	40,320
Threshold	2
Scan Start Delay	3 minutes
Ion Source Temp.	200°C
Electron Multiplier Voltage	per daily tuning (2000 to 2600 volts)
Injector Temp.	275°C
Separator Temp.	275°C
Transfer Line Temp.	250°C

We used the Hewlett Packard 5985A gas chromatograph/mass spectrometer/data system in our work.

Direct Insertion Mass Spectroscopy

One microliter of the sample, as 10% in toluene, is evaporated in the sample holder of the mass spectrometer direct insertion probe accessory. After being passed through the vacuum lock into the mass spectrometer, the sample is kept at 50°C for one minute, then heated at 10°C/minute to 250°C. [Based on the analytical work, there is evidence that the programmed heating rate was not the actual heating rate - that there was an overriding effect from the source temperature of 350°C.]

INORGANIC ANALYSES

Optical Emission Spectroscopy

Oil samples were prepared for analysis using a dry-ashing technique employing concentrated nitric acid containing a known quantity of gallium as an ashing aide. Gallium was dissolved in the nitric acid to provide a collector for the ashes formed from the metals in the oil and to serve as a common matrix when analyzed by DC arc excitation. The need for this became apparent after initial ashing established low levels of residue (<<1 milligram/gram) in the oils.

In the procedure that has been established 6 or more 5 gram samples are simultaneously ashed in 100 ml vycor evaporating dishes to which 5 ml of concentrated HNO_3 containing enough gallium to yield approximately 50 mg of Ga_2O_3. This results in a 100X concentration of metal residues in the oil and thus enables measurement of a considerable number of elements at the 0.1 ppm level and most of the remaining elements at 1 ppm concentrations.

The samples are compared against synthetic standards of Ga_2O_3 through a range of 0.1 to 10 ppm. To measure elements that exceed the 10 ppm limit it is necessary to redilute the Ga_2O_3 metal residue mix by a factor of 10. This is done in a lithium carbonate-graphite matrix and compared against like matrix standards. Thus the actual range of optical emission measurement is 0.1 to 100 ppm excepting vanadium for which a special set of standards have been made extending the capability to 400 ppm.

Twenty-eight elements are measured with an accuracy objective of $\leq\pm$ 25% for these elements >10 ppm and a range of 1/2 to 2X for analyses at the 1 ppm level.

Atomic Absorption Spectroscopy

The most basic element in this approach is a stable source, the hollow cathode lamp which is coupled to two other elements consisting of a burner system for the generation of a supply of neutral atoms and a monochromator plus detector to isolate and measure signal changes.

Solutions of a specific element are nebulized through the burner flame front where they are atomized and absorb energy from light emanating from the hollow cathode lamp. The resulting change in the signal strength of the lamp is monitored by the monochromator/detector system. By calibration with known quantities accurate measurements can be made of unknown materials.

Samples were prepared for measurement by digestion of approximately 5 grams of oil in vycor or platinum dishes and, after charring, finally igniting at 600°C; the ashes were dissolved in hydrochloric acid to a final volume of 25 milliliters containing 2% hydrochloric acid.

The measurements for Fe, Ni, and V are made using the parameters as outlined in the following table.

ANALYTICAL CONDITIONS USED ON THE VARIAN-TECHTRON AA6

ATOMIC ABSORPTION SPECTROPHOTOMETER

	ELEMENT		
Conditions	Fe	Ni	V
Wavelength	2483	2320	4379
Bandpass in nanometers	0.2	0.2	0.1
Photomultiplier Voltage	408	480	450
Flame type	air/acetylene	air/acetylene	nitrous oxide/ acetylene
Hollow cathode current in milliamperes	8	8	No hollow cathode/in the emission mode

ANALYTICAL CONDITIONS USED ON THE VARIAN-TECHTRON AA6

ATOMIC ABSORPTION SPECTROPHOTOMETER (Cont.)

Readout	Absorbance, 3 sec integration	Absorbance, 3 sec integration	emission, 3 sec. integration
Standards concentration range micrograms/milliliter	1-10	1-10	10-100

Arsenic and Selenium Analyses

An apparatus similar to one suggested by McDaniel et. al.[1] is used for the determination of As and Se by the hydride formation. The oil samples are decomposed by a fuming nitric, sulfuric, and perchloric acid mixture under reflux. The hydrides are liberated by sodium borohydride, separated in a liquid nitrogen - cooled trap (for arsenic) or led into a balloon reservoir (for selenium) and introduced into a hydrogen-nitrogen flame of an atomic absorption spectrophotometer.

The sensitivities, especially for arsenic, was improved considerably over other analytical methods tried. The reproducibility for both elements was excellent. Recoveries for both elements, using a synthetic standard prepared with Conostan base oil and containing 10 ppm arsenic and 10 ppm selenium are listed below:

RECOVERY OF As AND Se

Taken (each)	Found (As)	Found (Se)
10 μg/g	9.5	9.6
10 "	9.6	10.0
10 "	9.6	9.8
10 "	9.6	10.0

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[1] McDaniel, et.al. in "Concentration and determination of Se from environmental samples", Anal. Chem. 48, 14 (1976) 2240-2243

Miscellaneous Inorganic Analysis Methods

In response to the needs of the contract, the following inorganic analytical methods find lesser usage.

Oxygen Bomb Method

The oxygen bomb method is used for the determination of sulfur; chloride, bromide and iodide (calculated as chloride); and fluoride.

Sulfur Analysis

The total sulfur is determined after decomposition of a maximum of 1 gram of sample in an oxygen bomb (Parr, model 1108) using 5 ml of a 5% solution of Na_2CO_3 as absorbant and 35 atmospheres of oxygen pressure.

The products of combustion are washed along with the rinsings into a beaker, acidified with 1:1 HCl and 2 ml excess added. The solution is filtered and the sultate determined gravimetrically as $BaSO_4$.

When the available sample size is in the milligram or microgram range or the sulfur content at the trace level, a spectrophotometric method (Anal. Letters, 10(12), 985-996 (1977) is used after the oxygen bomb decomposition.

Chloride, Bromide, Iodide (Calculated as Chloride)

Four-tenths of one gram of sample is burned in the oxygen bomb under 30 atmospheres of oxygen. The bomb contains 5 ml of 5% Na_2CO_3 solution as absorbant. The carbonate solution is evaporated to a low volume and then distilled from sulfuric acid into 15 ml of 5% NaOH solution. The acidified NaOH solution is then titrated potentiometrically with 0.01 N $AgNO_3$.

Fluoride Analysis

The firing of the sample in the bomb is as above. The carbonante solution is evaporated to a low volume and acidified with sulfuric acid to pH5. TlSAB buffer is added and fluoride determined by the Orion specific ion electrode.

Kjeldahl Method for Nitrogen

The standard micro Kjeldahl procedure is used with the following conditions - sample size is 0.1 to 0.2 grams; the reaction mixture consists of 2 grams K_2SO_4, 1 Hengar pellet, 10 ml H_2O and 10 ml H_2SO_4.

SECTION 6

DISCUSSION

SAMPLE PROCUREMENT AND DISPOSITION

Forty-one residual fuel oil samples were obtained from various oil company sources during the course of this contract. There was great difficulty in obtaining these materials and their procurement is considered a significant accomplishment of this contract. The last of the samples was not received until November 1978. Due in part to such delays, not all samples fell into the examination program. Table 1 lists the samples received. Table 2 lists all sample data provided by the suppliers of the materials.

Twenty-eight of the residual fuel oils have been accepted into the U.S. Environmental Protection Agency/Department of Energy Fossil Fuels Research Materials Repository at Oak Ridge, Tennessee. Correlation between repository numbers and Westinghouse sample numbers are also given in Table 1.

As a result of our ability to obtain these samples, the project officer requested, as an extension of the contract, that we procure various samples of crude oil for trans-shipment to EPA. Accordingly, samples representative of oil production from 14 geographical areas were forwarded to EPA. These now reside in the above repository.

SAMPLE PREPARATION

The as received residual fuel oils are materials consisting of hundreds of individual organic compounds in different classes. While a sample en toto may not show mutagenicity, there could conceivably be component enhancement in the environment leading to substances of concern. And before attempting analysis by even such a powerful tool

Table 1

RESIDUAL OIL SAMPLES RECEIVED

W Sample No.	Repository Sample No.	Source	Sample Description	Refinery	Sample Code	Quantity	Date Received
1	5401	Shell	#6 High Sulfur Fuel Oil		41010	5 gal	Oct. 1977
2	--	Exxon	#6 Fuel Oil U.T.P. @ Palmetto		Dow Heater #2	1 qt	Nov. 1977
3	5402	Exxon	#6 Fuel Oil - 2.9% S		SX2432	1 gal	Dec. 1977
4A-E	5403	--	--		--	5x1 gal	Dec. 1977
5A,B	5404	Gulf	#6 Fuel Oil	Philadelphia, PA	LS8386	2x5 gal	15 Aug. 1978
6	5405	Exxon	#6 Hvy. Fuel Oil, 0.3% S (Caribbean Product)			5 gal	18 Aug. 1978
7	5406	Exxon	#6 Hvy. Fuel Oil, 2.9% S (Domestic Product)			5 gal	18 Aug. 1978
8	5407	Marathon Oil Co.	#6 Oil		Tank 300-8	1 gal	18 Aug. 1978
9	5408	Marathon Oil Co.	#6 Fuel Oil	Robinson, IL	RB-359	5 gal	23 Aug. 1978
10	5409	Am. Pet. Co.	#6 Fuel Oil	Port Arthur, TX	19798	5 gal	24 Aug. 1978
11A,B	5410	Gulf			TS 1922	2x5 gal	25 Aug. 1978
12A,B	5411	Gulf			TS 1923	2x5 gal	25 Aug. 1978
13	5412	ECI (Energy Cooperative Inc.)	#5 Fuel Oil			5 gal	28 Aug. 1978
14	5413	ECI	#6 Fuel Oil, Low Sulfur			5 gal	28 Aug. 1978
15	5414	ECI	#6 Fuel Oil, Medium Sulfur			5 gal	28 Aug. 1978
16	5415	ECI	#6 Fuel Oil, High Sulfur			5 gal	28 Aug. 1978
17	5416	Continental Oil Co.	#6 Fuel Oil		WR 4009	5 gal	30 Aug. 1978
18	5417	Boron Oil Co.	#6 Fuel Oil		LIR 513	5 gal	30 Aug. 1978
19	5418	Exxon	#6 Low Sulfur Fuel Oil	Baytown	SX 2674	5 gal	5 Sept. 1978
20	5419	Exxon	#6 Heavy Fuel Oil	Baytown	SX 2673	5 gal	5 Sept. 1978
21	5420	Conoco	#6 Fuel Oil		8576 DE 78-345	5 gal	7 Sept. 1978
22	5421	Sohio	#6 Heavy Fuel Oil	Toledo	TR 5996	5 gal	7 Sept. 1978
23	5422	Shell	#6 Fuel Oil	Martinez	41040	5 gal	7 Sept. 1978
24	5423	Shell	#5 Fuel Oil	Martinez		5 gal	7 Sept. 1978
25	5424	Cities Service	Decant Oil - H8	Lake Charles	S-248-78	5 gal	13 Sept. 1978
26	5425	Cities Service	#6 Fuel Oil	Lake Charles	S-249-78	5 gal	13 Sept. 1978
27	5426	Shell	#6 Fuel Oil	Houston-Deer Park	41010	5 gal	20 Sept. 1978
28	5427	Shell	#6 Fuel Oil	Norco, La.	QC 4398	5 gal	25 Sept. 1978
29	5428	Ashland	#6 Fuel Oil		KE 4-12-116	5 gal	25 Sept. 1978
30	--	Arco	#6 Fuel Oil	Philadelphia	40210	5 gal	5 Oct. 1978
31	--	Arco	#6 Fuel Oil	Philadelphia	40211	5 gal	5 Oct. 1978
32	--	Arco	#6 Fuel Oil	Philadelphia	40212	5 gal	5 Oct. 1978
33	--	Koch Ref. Co.	#6 Fuel Oil	St. Paul, Minn.	Tank #37	5 gal	10 Oct. 1978
34	--	Arco	#6 Fuel Oil, High Sulfur	Houston		5 gal	12 Oct. 1978
35	--	Arco	#6 Fuel Oil, Low Sulfur	Houston		5 gal	12 Oct. 1978
36	--	Chevron	#6 Fuel Oil			5 gal	26 Oct. 1978
37	--	Chevron	#6 Fuel Oil	Perth Amboy		5 gal	7 Nov. 1978
38	--	Chevron	#6 Fuel Oil, High Sulfur	Pascagoula		5 gal	9 Nov. 1978
39	--	B.P.	#6 Residual Fuel Oil	Marcus Hook		5 gal	13 Nov. 1978
40	--	B.P.	#6 Residual Fuel Oil	Marcus Hook		5 gal	13 Nov. 1978
41	--	Chevron	#6 Fuel Oil, Bunker	El Segundo		5 gal	16 Nov. 1978

Table 2

PHYSICAL PROPERTIES OF RESIDUAL OIL SAMPLES

W Sample No.	Source	Sp. Grav. ° API	Flash °F	Pour Point °F	Sulfur %	V ppm	Na ppm	Visc.		Carbon Res. (0524)	Asphalt % I P 143	Ash % D-482
								@ 100°F	@ 122 F Sec.			
1	Shell-Deer Park	9-11	180	+35	2.5	100	30	-	150-250	-	-	0.06
2	Exxon-Palmetto	15.9	-	-	1.45	-	-	-	245	-	-	-
5	Gulf-Phila.	14.6	215	+80	0.60	12	-	-	221	7.51	0.70	0.037
6	Exxon-Bayway				0.3							
7	Exxon-Bayway				2.9							
10	Fina-Dallas	7.5	184	60	2.19	-	-	-	233	12.8	-	0.107
11	Gulf-Toledo	17.0	210	45	1.06	-	-	-	46.4	-	-	-
12	Gulf-Toledo	14.6	216	35	1.43	-	-	-	194	-	-	-
13	Energy Cooperative	18.5	180+	-10	0.8	-	-	240 sus	-	-	-	-
14	Energy Cooperative	14.4	230+	+35	0.84	-	-	-	179	-	-	-
15	Energy Cooperative	15.1	230+	+35	1.36	-	-	-	198	-	-	-
16	Energy Cooperative	11.9	230+	+40	1.66	-	-	-	188	-	-	-
19	Exxon - Baytown	-	190-230	0 to +25	0.60-0.69	10-20	5-25	-	60-80	15	6%	0.03
20	Exxon - Baytown	12-13.5	160-170	10-35	2.2-2.4	60-500	20-100	-	185-186	-	14.0	0.05-0.09
23	Shell - Martinez	9.0	200	+35	1.5	100	60	-	150-225	-	-	0.06
24	Shell - Martinez	11.0	200	+20	1.5	80	50	-	30	-	-	0.06
25	Cities Service - Lake Charles	0	194	+75	2.0-2.2	-	8.93	895.3 (° FCS)	-	9.75	3.58	0.07-0.10
26	Cities Service - Lake Charles	5 min.	150	+60	1.0 min.	100	75	-	100 min. 300 max.	-	-	0.10 max.
27	Shell - Houston	9-11	180	+35	2.5	100	30	-	150-250	-	-	0.06
28	Shell - Norco	10.0	180	+50	0.95	10	30	-	150-250	-	-	0.02
33	Koch	9.9	224	30	1.8	-	-	-	100	-	-	-
34	Arco-Houston	12.7	200+	30	2.38	45	17	900	196	-	-	0.016
35	Arco-Houston	15.0	200+	75	0.93	<10	10	1100	240	-	-	0.0015

as a gas chromatograph/mass spectrometer/data system (GC/MS/DS) it is necessary to simplify the sample into fractions of basically different composition. This was done by means of column chromatography and solvent partitioning.

Column Chromatography

Column chromatography consists of using a series of solvents of increased polarity to move sample components of increasing polarity through a vertical column of adsorbant. The more polar the adsorbant, the better the sample resolution but the less total sample recovery. Results of some of the early column chromatography work illustrate these effects as in Table 3.

In handling the problem of column polarity/recovery it could be advantageous to have a highly polar column material (for sample resolution) that could be made less polar at the end of a separation (for sample recovery). Accordingly, 5% aqueous sodium sulfate (Na_2SO_4) was cast onto 100 grams of florisil adsorbant and the mixture dried overnight at 110°C. A weighed sample of about 4 grams of a fuel oil was cast from methylene chloride (CH_2Cl_2) onto 25 grams of the prepared adsorbant and dried at 110°C for 16 hours. The sample mix was then layered on top of a column consisting of the remaining 75 grams of florisil/Na_2SO_4. The sample was eluted with various solvents, then with water (to remove Na_2SO_4), then again with various solvents. The data obtained is summarized in Table 4. It would be difficult to compare total recovery with previous work involving this sample (i.e. the data of Table 3) since this latter work involved the 15% hexane insolubles/CH_2Cl_2 solubles not involved in the earlier work. It can be seen that solvent elution recovered 4.77% additional sample after column polarity was changed by washing out the Na_2SO_4.

Further work indicated a minimum amount of information was obtained off column after the elution of the Na_2SO_4 with water and further solvent treatment. Fractions 4-6-14 and 4-6-15 as in Table 4 did not in fact

Table 3

COLUMN CHROMATOGRAPHY OF SHELL FUEL OIL NO. 6
(Westinghouse Sample No. 1)

Developing Solvent	Florisil	Alumina	Molecular Sieve 13X
Hexane	62.3	39.3	29.5
10% Acetone/Hexane	19.0*	33.0	23.0
50% Acetone/Hexane	1.2	2.5	2.6
Acetone	2.4	1.3	1.3
Methylene Chloride	--	<1.0	<<1.0
Total Sample Recovery**	100.2	91.4	71.7

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*15% Acetone/Hexane

**Includes 15.3% hexane insoluble portion of sample

Table 4

FUEL OIL NO. 6 THROUGH Na_2SO_4/Florisil Column

Fraction	Developing Solvent	Sample Recovery	Comments
4-6-1	Hexane	29.66%	
4-6-2	Hexane/1% Acetone	9.44	
4-6-3	Hexane/2% Acetone	19.44	
4-6-4	Hexane/5% Acetone	6.61	83.23% of the sample
4-6-5	Hexane/10% Acetone	5.89	recovered from Na_2SO_4 -
4-6-6	Hexane/25% Acetone	5.29	containing column
4-6-7	Hexane/50% Acetone	2.35	
4-6-8	Acetone	0.93	
4-6-9	Methylene Chloride	1.52	
4-6-10	Methanol	2.10	
			Na_2SO_4 washed from column
4-6-11	Water	0.11	
4-6-12	Acetone	1.97	4.77% additional sample
4-6-13	Methylene Chloride	1.50	recovered from Na_2SO_4 -
4-6-14	Methanol	0.43	free column
4-6-15	Toluene	0.76	
4-6-16	Toluene	0.90	Column packing removed and extracted with boiling toluene
4-6-16	Dioxane	0.41	Column packing extracted with dioxane at room temperature
TOTAL SAMPLE RECOVERY		89.31%	

appear to contain any analytical volatiles when tested later. Because the florisil/Na_2SO_4 system did seem to be effective, the method was retained but with elimination of the water wash and subsequent steps. All column material from all sample column separations have been retained, and could be subjected to additional treatment.

The solvent elution system finally chosen was based on that used by Farcasiu.[1] Solvent changes were made in the direction of materials with less potential environmental impact (toluene for benzene, methylene chloride for chloroform, etc.). Quantities of solvent to be used for elution were determined by the data of Figure 1. After initial work, hexane with 10% diethyl ether was eliminated as an eluant (i.e. - seven solvent systems were used to collect seven fractions of a sample).

Thirty-two residual fuel oil samples were subjected to column chromatography pre-separation. The data is presented in Table 5. An attempt is made to "pattern" residual fuel oils in Table 6 based on their "aliphatics" content (i.e. - by the relative proportion of column chromatography fraction #1). Some of the possibilities suggested by the "average" data of Table 6 are:

1. Residual oils can have an "aliphatics" (fraction 1) content of about 15 to 70%.
2. Most residual oils have an aliphatics content in the 20 to 35% range.
3. In oils with fraction 1 contents of 15 to 35%, the contents of fractions 2 through 7 remain rather constant.
4. Oils with fraction 1 contents of 50 to 65% have contents of fractions 3 through 7 which suggest they may be "Fraction 1, 15 to 35%" oils containing an aliphatic diluent.

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[1] M. Farcasiu, Fractionation and Structural Characterization of Coal Liquids, Fuel, Vol. 56, p. 9-14 (January 1977).

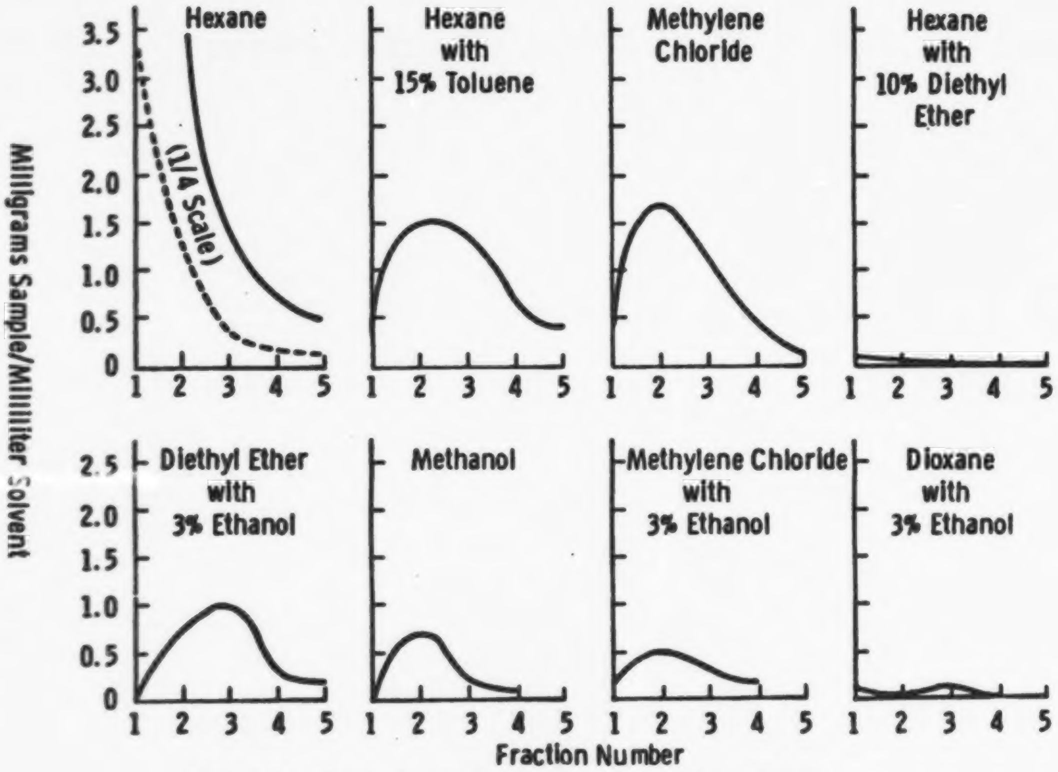

Figure 1. Sample Recovery with the Various Solvent Systems

Table 5

COLUMN CHROMATOGRAPHY OF FUEL OILS

	Percent Recovery of Sample							
Sample	Hexane	Hexane with 15% Toluene	Methylene Chloride	Diethyl Ether with 3% Ethanol	Methanol	Methylene Chloride with 3% Ethanol	Dioxane with 3% Ethanol	Total Recovery
Shell #6 Fuel Oil, High Sulfur #41010	20.9	19.9	18.6	12.5	5.28	4.97	0.44	82.6
Exxon #6 Fuel Oil SX 2432	15.6	16.4	16.6	14.0	4.26	5.17	0.74	72.7
	15.5	14.3	17.4	15.0	3.98	4.76	1.06	72.0
Gulf #6 Fuel Oil LS 8386	33.9	16.8	13.9	12.3	5.08	4.72	0.31	87.0
Exxon #6 Heavy Fuel Oil, 0.3% Sulfur (Caribbean Prod.)	61.1	17.2	6.40	2.39	2.44	0.65	0.16	90.3
ECI #5 Fuel Oil	29.8	17.2	7.73	9.17	3.60	2.81	0.20	70.5
ECI #6 Fuel Oil Low Sulfur	29.4	23.9	14.7	11.2	3.65	3.49	0.51	86.8
ECI #6 Fuel Oil Medium Sulfur	24.9	21.1	13.6	10.5	3.88	3.58	0.51	78.1
ECI #6 Fuel Oil High Sulfur	25.4	18.9	17.8	12.3	4.71	4.84	1.40	85.4
Exxon #6 Fuel Oil UTP @ Palmetto	34.5	18.7	16.4	8.31	4.42	4.20	0.30	86.8
Sample 4D (Reclaimed Oil)	33.9	14.4	12.1	11.1	4.53	4.51	0.41	81.0
Exxon #6 Hvy. Fuel Oil 2.9% Sulfur (Domestic Prod.)	25.7	16.1	19.6	11.6	5.21	5.23	0.41	83.9
Marathon Oil Co. #6 Oil, Tank 300-8	63.9	13.9	7.26	3.58	1.98	1.58	0.31	92.5
Marathon Oil Co. #6 Fuel Oil Sample RB-359	35.7	13.8	14.9	13.8	3.25	3.38	0.69	85.5
RIMA #6 Fuel Oil Port Arthur	22.8	21.6	18.9	11.4	5.62	4.60	0.82	85.7
Gulf Oil Co. TS-1922	23.7	16.9	12.0	11.9	3.69	3.92	0.37	72.5
Gulf Oil Co. TS-1923	22.4	17.3	16.6	12.6	3.52	4.59	0.87	77.9
Continental Oil Co. #6 Oil WR 4009	28.5	18.1	21.2	8.90	4.99	4.07	0.43	86.2
Boron Oil Co. #6 Oil LIR #513	35.7	14.3	16.6	10.2	3.03	3.70	0.38	83.9
Exxon #6 Low Sulfur Fuel Oil SX #2674	51.3	18.9	11.3	5.71	2.42	2.01	0.19	91.8
Exxon #6 Hvy. Fuel Oil 2.19% Sulfur SX #2673	21.3	17.4	20.1	11.9	4.28	5.89	0.38	81.3

Table 5 (Cont)

	Percent Recovery of Sample							
Sample	Hexane	Hexane with 15% Toluene	Methylene Chloride	Diethyl Ether with 3% Ethanol	Methanol	Methylene Chloride with 3% Ethanol	Dioxane with 3% Ethanol	Total Recovery
Sample 4D (Reclaimed Oil)	33.9	14.4	12.1	11.1	4.53	4.51	0.41	81.0
Exxon #6 Hvy. Fuel Oil 2.9% Sulfur (Domestic Prod.)	25.7	16.1	19.6	11.6	5.21	5.23	0.41	83.9
Marathon Oil Co. #6 Oil, Tank 300-8	63.9	13.9	7.26	3.58	1.98	1.58	0.31	92.5
Marathon Oil Co. #6 Fuel Oil Sample R8-359	35.7	13.8	14.9	13.8	3.25	3.38	0.69	85.5
RINA #6 Fuel Oil Port Arthur	22.8	21.6	18.9	11.4	5.62	4.60	0.82	85.7
Gulf Oil Co. TS-1922	23.7	16.9	12.0	11.9	3.69	3.92	0.37	72.5
Gulf Oil Co. TS-1923	22.4	17.3	16.6	12.6	3.52	4.59	0.87	77.9
Continental Oil Co. #6 Oil WR 4009	28.5	18.1	21.2	8.90	4.99	4.07	0.43	86.2
Boron Oil Co. #6 Oil LIR #513	35.7	14.3	16.6	10.2	3.03	3.70	0.38	83.9
Exxon #6 Low Sulfur Fuel Oil SX #2674	51.3	18.9	11.3	5.71	2.42	2.01	0.19	91.8
Exxon #6 Hvy. Fuel Oil 2.19% Sulfur SX #2673	21.3	17.4	20.1	11.9	4.28	5.89	0.38	81.3
Continental Oil Co. #6 Fuel Oil Code B576	31.5	19.3	19.5	9.95	4.34	3.52	0.42	88.5
SOHIO #6 Hvy. Fuel Sample Order NO. 78-5996	28.5	21.5	14.6	9.72	3.65	3.45	0.37	81.8
Shell #6 Fuel Oil #41040 (Martinez)	24.3	16.8	11.1	11.6	7.10	3.48	0.49	74.9
Shell Heavy #5 Fuel Oil #40140 (Martinez)	19.0	18.3	13.9	9.47	8.08	4.09	0.51	73.4
Cities Service Decant Oil H-8 Sample #5-248-78	17.9	17.7	35.3	6.62	6.58	4.78	0.52	89.4
Cities Service #6 Fuel Oil Sample #5-249-78	37.0	16.7	15.3	10.3	3.77	2.78	0.37	86.2
Shell #6 Fuel Oil #41010 (Houston)	23.1	15.4	17.7	12.6	4.34	4.66	0.47	78.3
Shell #6 Fuel Oil QC #4398	25.4	18.0	23.2	11.2	4.83	3.81	0.35	86.8
Ashland Oil Co. #6 Fuel Oil KE 4-12-116	25.3	19.0	17.1	8.42	4.69	2.71	0.28	77.5

Table 5 (Cont)

	Percent Recovery of Sample							
Sample	Hexane	Hexane with 15% Toluene	Methylene Chloride	Diethyl Ether with 3% Ethanol	Methanol	Methylene Chloride with 3% Ethanol	Dioxane with 3% Ethanol	Total Recovery
Arco AP-101-666 Sample #40210	69.0	18.9	6.30	1.79	1.32	0.43	0.10	97.9
Arco AP-102-670 Sample #40211	51.2	17.7	13.6	7.77	3.30	1.63	0.21	95.4
Arco AP-103-415 Sample #40212	67.7	19.2	7.15	1.80	1.69	0.21	0.005	98.0

Table 6

POSSIBLE PATTERNS AS SEEN IN THE COLUMN CHROMATOGRAPHY OF RESIDUAL OIL SAMPLES

Range of Sample Recovery as Fraction 1	No. of Oils in Set	Percent of Sample Recovery as Fraction No.												Percent Total Sample Recovery	
		2		3		4		5		6		7			
		Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range
15-20%	2	16.9	15.4; 18.3	15.5	13.9; 17.0	12.0	9.47; 14.5	6.10	4.12; 8.08	4.53	4.09; 4.97	0.71	0.51; 0.90	72.9	72.4; 73.4
20-25%	8	18.3	15.4-21.6	16.1	11.1-20.1	11.9	10.5-12.6	4.72	3.53-7.10	4.46	3.48-5.89	0.55	0.37-0.87	78.9	72.5-85.7
25-30%	8	19.1	16.1-23.9	17.0	7.73-23.2	10.3	8.42-12.3	4.42	3.60-5.21	3.80	2.71-5.23	0.49	0.20-1.40	82.3	70.5-86.8
30-35%	6	16.2	13.8-19.3	15.6	12.1-19.5	10.9	8.31-13.8	4.11	3.03-5.08	4.01	3.38-4.72	0.42	0.30-0.69	85.5	81.0-88.5
35-40%	1	16.7	—	15.3	—	10.3	—	3.77	—	2.75	—	0.37	—	86.2	—
50-55%	2	18.3	17.7; 18.9	12.5	13.6; 11.3	6.74	7.77; 5.71	2.86	3.30; 2.42	1.82	1.63; 2.01	0.20	0.19; 0.21	93.6	91.8; 95.4
60-65%	2	15.6	13.9; 17.2	6.83	7.26; 6.40	2.99	3.58; 2.39	2.21	1.98; 2.44	1.12	0.65; 1.58	0.24	0.16; 0.31	91.4	90.3; 92.5
65-70%	2	19.1	18.9; 19.2	6.73	6.30; 7.15	1.80	1.79; 1.80	1.51	1.32; 1.69	0.32	0.21; 0.43	0.05	0.005; 0.10	98.0	97.9; 98.0

5. If the higher aliphatic content oils (50 to 70% as fraction 1) are achieved by dilution of refinery residuals, then the diluent is of a composition that does not change the absolute amount recovered as fraction 2 when compared to a "15-35% as fraction 1" oil.
6. If dilution has not been used with any of the oils investigated, the quantity in fraction 1 (either alone or as compared to any other fraction) may be a measure of some other feature of the oil, such as perhaps, its source. Comparison of fraction 2 with any other fraction would be more limited in usefulness. Comparison among fractions 3 through 7 would not be expected to yield any information in this context.

It would be interesting to make an evaluation of the trend in total sample recovery (i.e., the final two columns in Table 6). The average recovery appears to follow average polarity of the samples. However, one major unknown is the degree of insolubility of the as-received samples in any of the solvent systems used for column chromatography. It is expected that "% insolubles" would not be a constant value with all samples.

All of the above comments would be more credible if individual fraction recoveries did not range so greatly about the average. A large number of samples would have to be tested before there would be reasonable confidence that the above supposed trends are real.

One of the 32 oils subjected to column chromatography, Cities Service Decant Oil H-8 (W Sample No. 25), is not included in the data of Table 6. This is done for several reasons:

1. The column chromatography data on this oil is in great variance with the patterns of Table 6.
    a. The "fraction 3" at 35% is about double that of the usual oil sample.

   b. The total sample recovery from the column (89.4%) is high for an oil with an "aliphatics" content in the 15 to 20% range.

2. The infrared pattern of the as-received oil has an unusually weak "long-chain aliphatic hydrocarbon" peak at 720 cm^{-1}.

   a. Infrared spectra of fraction 1 from this oil show the fraction to be an alipatic hydrocarbon with shorter than "normal" chain length.

3. The infrared pattern of the as-received oil has a prominent peak at 1032 cm^{-1}. This position is normally associated with the presence of sulfur. However, the peak appears relatively stronger than with other samples of the same nominal sulfur content (approximately 2%). [It will also be noted later that this oil is the lowest in vanadium of any of the first 25 samples - 0.3 ppm.]

Solvent Partitioning

In order to present the residual fuel oil to bioassay by yeast, *E. coli*, and Ames test, it is necessary to disperse it into a solvent compatible with the biological test agents. Two applicable solvents of interest are dimethyl sulfoxide (DMSO) and p-dioxane. Early work was performed with DMSO but this appeared to be hampered by a relatively low sample solubility in this solvent (about 45% for W sample #4). Dioxane on the other hand was capable of dissolving 97.24% of W sample #4 and appeared to present no compatibility problems as a bioassay medium. It was later found that on repeated exposure to air, dioxane becomes toxic to bioassay tester organism (presumably through peroxide formation) and must be kept under an inert atmosphere. All bioassays of as received residual fuel oils and of column chromatography (no partitioning) fractions were performed in dioxane.

There is another important observation on the use of dioxane as a solvent for partitioning of residual fuel oils. Based on the limited investigation possible under work priorities, a few as received fuel oil samples were extracted with dioxane. The dioxane insolubles (varying, but less than 7.5 wt % of the original sample in all cases) were then extracted with toluene. The toluene then left less than 0.2% of the original sample as insolubles. Of importance is the experimental experience that the dioxane insolubles, toluene solubles, effect a large concentration of the original iron, nickel and vanadium contents of the oil. In addition to being a potential means of concentrating metal compounds into a discrete fraction without altering their composition, there is the important fact that these metals would have to be present as some type(s) of metallo-organic rather than as an inorganic occlusion in order to be soluble in toluene. Such metallo-organics could conceivably be more biologically "active" than a straight inorganic expression of a metal. Data that has been obtained in these contexts is discussed later in this section under inorganic analyses.

The most important partitioning work done under this contract involved the use of DMSO. As seen in Table 5 the aliphatic contents (i.e. - fraction 1) of the 32 residual fuel oils that were subjected to column chromatography ranged from 15 to 70%. These aliphatics serve to dilute the sample without any known contribution to concern for the environment. Our work through mass spectral and infrared analyses has shown that organic material of interest is essentially if not completely dissolved away from these aliphatics by DMSO. Using our residual fuel oil with greatest potential mutagenicity properties (Westinghouse sample #16) we made an important observation -- DMSO solubles of this oil are mutagenic; DMSO insolubles are not. Because of the complexity and "individuality" of residual fuel oils, a concept such as above would have to be thoroughly researched. However, this DMSO partitioning appears to have led to our most powerful sample prefractionation scheme - DMSO partitioning/column preseparation.

Dimethylsulfoxide Partitioning/Column Preseparation

This technique consists of subjecting an as received residual fuel oil to DMSO partitioning followed by our column chromatography of the DMSO solubles and of the DMSO insolubles. If usage with a statistically valid number of samples showed that fractions from the DMSO insolubles were not mutagenic, work on DMSO insolubles would be eliminated. Applied to our "most mutagenic" sample (Westinghouse sample #16) column separation of DMSO solubles produced fractions, one of which was the most mutagenic seen in this study - 16 times the level of mutagenicity seen with any previous sample or fraction. The 7 column separation fractions of DMSO insolubles, oil #16, were all non-mutagenic by Ames test. DMSO partitioning followed by column chromatography should be the technique used with any future work of this type.

BIOASSAY RESULTS

Throughout the bioassay results discussion, the Westinghouse sample number is preceded by RO (Residual Oil) designation.

As Received Residual Fuel Oils

Test with *Saccharomyces* and *E. coli*

The Litton Bionetics, Inc. performed bioassays on twenty-six as-received residual oils dissolved in p-dioxane.

The test compounds were examined for mutagenic activity in a series of *in vitro* microbial assays employing *Saccharomyces cerevisiae* D4 and *E. coli* WP_2 uvr A^- indicator organisms. The compounds were tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats.

The compounds were tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effect at the highest dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of these compounds was from 0.005 µl to 10 µl per plate.

The results of the tests conducted on the compounds in the absence of a metabolic activation system were all negative.

The results of the tests conducted on the compounds in the presence of a rat liver activation system were all negative.

The test compounds RO #1 to #26 and RO #3, fraction 1-9, did not demonstrate genetic activity in any of the assays conducted in these evaluations and were considered not mutagenic under these test conditions.

The results were carefully rechecked, but no positive response could be discerned in any of the as-received oil residues. The LBI results are presented in their full form in Appendix A.

Ames Test by Stanford Research International, Inc.

The Ames test results of the 26 residual oils as-received and solubilized (extracted) with p-dioxane and analyzed by SRI are presented in their full form in Appendix B, with the appropriate SRI evaluation accompanying the data. The SRI data was carefully analyzed and conclusions were drawn as to the mutagenic response prior to reading the SRI conclusions. From this it became evident that there are differences between W and SRI in classifying a compound a mutagen.

The premise for classifying a substance a mutagen in the Ames test requires that certain criteria are first met.

1. To compensate for growth irregularities and toxicity for each concentration, the revertant increase must be twice that of the spontaneous revertant count. In our evaluations we have employed the test revertant over control revertant ratio. If this ratio R is 3.0 or higher, that concentration is considered positive.

2. Also a dose related response curve must be present to call a substance positive in a test.

3. The results must be repeatable in two separate tests on two separate dates. If both tests are positive, the substance is then declared a mutagen.

In a summarized form our conclusions, based on the above criteria and the raw data versus the SRI conclusions are as follows for the 26 as received oils.

There was no mutagenic activity with or without metabolic activation in tester strain TA 1535. For that reason there will be no further discussion or inclusion of this strain in the tabulated results.

We agree with the submitted conclusions on 16 samples or 62% in that 11 (42%) are positive (RO #1,2,3,5,9,15,16,20,22,23,24) 4 (or 15%) are negative (RO #10,11,12,17) and RO #13 is questionable in response, i.e., only one out of two tests was positive, and then only with activation in TA 1538.

On four samples we considered mutagenicity questionable, while SRI calls RO #18,25,26 positive, but RO #8 negative. In addition we judge four samples (RO #6,7,19,20) to be negative while SRI considers the results indicative of possible weak mutagenic response. On samples RO #4 and 14 there is a complete disagreement. While SRI calls these two residual oils positive, we can find no justification for such a conclusion.

From Table 7 we observe that there is agreement on 16 samples; 11 positive (RO #1,2,3,5,9,15,16,20,22,23,24), one (RO #13) maybe, and four (RO #10,11,12,17) negative. In eight samples the variance is one of degree; three SRI positives are classified as maybe's by W; four SRI maybe's are called negative, and one negative SRI is a maybe in

Table 7

SRI AMES TEST DATA

(as Interpreted by W and by SRI)

RO#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Positive W	w	w	w		w				w						w	w				w		w	w	w		
Positive SRI	s	s	s	s	s				s					s	s	s		s		s		s	s	s	s	s
Maybe W								w					w					w							w	w
Maybe SRI						s	s						s						s		s					
Negative W				w		w	w			w	w	w		w			w		w		w					
Negative SRI								s		s	s	s					s									

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*All bioassay tables and figures retain the sample numbering of RO (Residual Oil) incorporated in the captions. The numerical designation of bioassay samples corresponds to the Westinghouse Sample Number. Only the prefix RO is added.

our evaluation. The real question is on samples RO #4 and RO #14. RO #4 has only one positive response, and that is at 5000 µg/plate without activation in TA 1535. This strain did not respond in any other samples. The same applies to RO #14, which SRI calls positive for mutagenesis, but W considers negative. Of four separate tests, none were positive. Only in TA 1538 with activation in the last (fourth) test was there indication of a possible dose response. The R value, however, reached only 2.6 at 500 and 1000 µg plate and "dropped" to 2.5 at 2500 µg plate. In total, there are 11 positive, 5 questionable and 10 negative samples according to W data interpretation. In contrast, SRI judges 16 samples positive, 5 each questionable or negative.

When the 11 positive samples are further analyzed, we find that four were positive without and with metabolic activation. With activation all samples responded to strain TA 1538 and TA 98, four to TA 1537, and two to TA 100. Thus we are dealing with frameshift mutagens in our oil residue samples. Rearranging the data in another manner, two samples with metabolic activation were positive for strains TA 1537, TA 1538, TA 98, and TA 100, two for TA 1537, TA 1538 and TA 98. No positive response was observed in TA 1535. Without activation two responded to TA 1537 and TA 1538, one to TA 1538 and TA 98, and one only to TA 1537. These responses are presented in table form as follows:

Table 8

SRI SAMPLE RESPONSE BY TESTER STRAINS

	No Activation			Activation			
RO #	TA 1537	TA 1538	TA 98	TA 1537	TA 1538	TA 98	TA 100
1	x			x	x	x	x
2				x	x	x	
3				x	x	x	
5					x	x	
9					x	x	
15					x	x	
16	x	x			x	x	
20	x	x			x	x	
22					x	x	
23					x	x	
24		x	x	x	x	x	x

No correlation could be established between the positive response and the source of the oil residue sample.

When the eleven samples, which meet the set forth criteria for a mutagenic substance are tabulated according to the highest recorded response (Table 9) the samples then can be ranked according to their potency (Table 10) and response in the tester strains.

From this potency rank and strain response several observations can be made.

1. All positive samples respond in TA 1538 and TA 98 with metabolic activation using S9 Mix; hence are frameshift mutagens.
2. Except for the fourth ranked sample, the top five also react without activation, however, the response is not uniform.
3. The five least active samples respond only with activation in strains TA 1538 and TA 98.

Ames Test by Westinghouse

The most mutagenically active residual fuel oil as reported by SRI, RO #24, was further studied by Westinghouse as a check on the bioassay results. The sample was dissolved in both DMSO and p-dioxane at 50 mg/ml of solvent and subjected to the Ames test at Westinghouse R&D Center. The results of this battery of tests are presented side-by-side with those by SRI in Table 11.

In the SRI report RO #24 is positive without activation for TA 1538 and TA 98 and with metabolic activation for all strains except TA 1535. The non-activation repeat test by W confirmed on the surface the SRI results and added TA 1535 and TA 1537 as positives. The validity of these results was immediately questioned when precipitate was observed in the aqueous media at high RO #24 concentration and also reported by SRI. This gave high Biotran II (automated) readings not consistent with visual observation of the plates. Therefore, in all questionable cases, the cumbersome, time consuming manual readings were

Table 9

HAZARDOUS SUBSTANCES IN RESIDUAL OIL
RESPONSE AMES TEST

Highest R (test over control revertants) Reported

	No Activation					With Activation (S9)				
Sample RO No.	TA 1535	TA 1537	TA 1538	TA 98	TA 100	TA 1535	TA 1537	TA 1538	TA 98	TA 100
1		45					7.3	4.1	5.1	3.2
2							3.3	5.1	3.4	
3							5.0	3.6	3.1	
4										
5								3.6	3.3	
6										
7										
8										
9								5.6	3.7	
10										
11										
12										
13										
14										
15								3.3	3.2	
16		3.5	3.2					6.4	8.1	
17										
18										
19										
20		4.2	4.6					3.8	4.9	
21										
22								4.8	3.8	
23								5.5	4.9	
24			4.0	3.8			3.3	12.0	10.6	3.1
25										
26										

Table 10

HAZARDOUS SUBSTANCES IN RESIDUAL OIL
AMES TEST
STRAIN RESPONSE OF POSITIVE SAMPLES

		No Activation			With Activation (S9)			
Potency Rank	Sample RO No.	TA 1537	TA 1538	TA 98	TA 1537	TA 1538	TA 98	TA 100
1	24		+	+	+	+	+	+
2	16	+	+			+	+	
3	1	+			+	+	+	+
4	2				+	+	+	
5	20	+	+			+	+	
6	3				+	+	+	
7	22					+	+	
8	23					+	+	
9	9					+	+	
10	5					+	+	
11	15					+	+	

also made. Using the manual counts no strain was positive without activation. The same procedure was then repeated with metabolic activation, careful background damping of the Biotran II, and manual counting whenever visual observation indicated the necessity for it. Using this procedure, the following strains were negative for mutagenicity with activation: TA 1535, TA 1537 and TA 100. TA 1538 and TA 98 were positive at the 2500 μg/plate concentration, but only barely on the borderline, although a dose response appears to be present.

When the SRI data is analyzed, the possibility of interference of the precipitate can be seen between the two test runs for each strain with RO #24. The best examples are TA 1538, no activation (test #1 negative, test #2 positive) and TA 100 with activation (test #1 positive, test #2 negative).

Two of the most "active" SRI residual oil samples were also checked for DNA damage with the E. coli Pol A^+/Pol $A1^-$ system. These were RO #16 and RO #24. This is very important in light of the fact that the yeast and E. coli WP_2 urv A^- were negative for all 26 samples. Both samples in p-dioxane at 500, 1000, and 2500 μg/plate levels and three plates per concentration were negative for DNA damage. This further demonstrates the need for a thorough re-evaluation of the Ames test results in order to obtain a true picture of the possible health hazards from these residual oils. From the manual test scores it was concluded that RO #24 is not mutagenic without activation, and only very weakly with activation in TA 98. Interference with oil precipitate on the plates at higher (500 μg and up) concentrations produces increased values with the automated colony counter. This was suggested as the cause of data variation in the results. Subsequent contact with SRI indicates that due to the precipitate on the plates, most plates had to be hand-counted by SRI. This negates the original theory that the automatic counting technique could be responsible for the data discrepancy.

Because of the importance of assessing the possible environmental impact of oil residues, the eleven SRI positive samples were retested by Westinghouse to verify their mutagenicity. One negative sample, RO #12 was added as an internal control. The SRI data clearly demonstrated that all positive samples respond in tester strains TA 1538 and TA 98, therefore, only these two strains were employed in the re-testing experiment. The results are presented in Table 12. The SRI data is in Appendix B.

To better visualize the W and SRI data comparison, bar graphs were prepared, Figure 2 to Figure 49 and data summarized in Table 13 for TA 1538, and in Table 14 for TA 98. Westinghouse data is given in Table 12.

Table 11

RESIDUAL OIL SAMPLE RO 24 AMES TEST RESULTS

The Ratio of Test over Control Revertants is Presented
to Permit Direct Comparison

		μg RO 24 added per plate					
		10	50	100	500	1000	2500
TA 1535							
No Activation	SRI dioxane	.7	.6	.8*	.9*	.8*	.5*
		1.0	1.2	1.2*	1.1*	1.2*	.9*
	W DMSO	.8	.7	.9	1.1*	1.7*	9.1*
	DMSO, manual						.8*
	dioxane	.6	1.1	.9	1.4*	3.5*	18.0*
	dioxane, manual						1.2*
Activation	SRI	.9	.6	.5*	.6*	.9*	.5*
		1.2	.8	.6*	.8*	.8*	1.0*
	W DMSO	.7	.9	.8	.7s	.6s	.7*
	dioxane	.9	.8	.9	.8s	.9*	1.2*
TA 1537							
No Activation	SRI dioxane	.9	1.3	1.0*	.8*	1.6*	2.0*
		1.1	1.0	.9*	.7*	1.2*	1.8*
	W DMSO	1.9	2.0	1.1	3.8*	3.4*	30.4*
	DMSO, manual	1.0	.9	.9	1.7*	1.8*	.8*
	dioxane	2.1	2.3	2.0	5.9*	9.1*	62.3*
	dioxane, manual	1.1	1.3	1.9	1.3*	1.6*	1.0*
Activation	SRI dioxane	1.5	2.1	2.5*	3.2*	3.7*	3.4*
		1.8	1.3	2.0*	3.1*	3.5*	3.2*
	W DMSO	1.2	1.1	1.2	1.5s	1.5s	1.4*
	dioxane	1.5	1.3	1.2	1.5s	.9*	1.5*
TA 1538							
No Activation	SRI dioxane	2.9	1.6	2.3*	2.8*	2.1*	1.4*
		4.1	6.4	6.9*	5.3*	4.4*	9.6*
	W DMSO	.9	.8	.9	1.1*	2.1*	15.1*
	dioxane	1.1	1.5	1.5	2.8*	8.1*	27.3*
	dioxane, manual	1.1	1.6	1.6	1.1*	.8*	.4*
Activation	SRI dioxane	2.5	4.7	5.6*	7.2*	7.9*	10.5*
		3.1	4.2	6.2*	8.5*	19.7*	5.8*
	W DMSO	1.3	1.6	2.0	1.8s	2.7s	3.4*
	dioxane	.7	1.1	1.7	2.5s	2.3*	2.8*

Table 11 (Cont)

		µg RO 24 added per plate					
		10	50	100	500	1000	2500
TA 98							
No Activation	SRI dioxane	.9	1.9	1.8*	2.5*	2.6*	3.8*
		1.3	2.2	3.3*	3.8*	3.1*	3.8*
	W DMSO	.7	.8	.8	1.3*	2.1*	11.9*
	dioxane	1.1	1.1	1.2	2.4*	5.0*	18.4*
	dioxane, manual	1.3	1.4	1.7	1.4*	1.7*	.9*
Activation	SRI dioxane	2.6	5.2	6.1*	8.1*	10.0*	10.8*
		2.4	5.7	6.8*	8.8*	9.6*	10.3*
	W DMSO	1.1	1.6	1.8	2.3s	2.3s	2.8*
	dioxane	1.2	1.3	1.6	2.0s	2.6*	3.4*
TA 100							
No Activation	SRI dioxane	1.0	1.0	1.1*	1.5*	1.3*	1.2*
		1.1	1.2	1.2*	1.3*	1.2*	1.2*
	W DMSO	.9	1.0	1.0	.8*	1.1*	1.9*
	dioxane	.9	.9	.9	.9*	1.1*	2.0*
Activation	SRI dioxane	1.4	2.5	2.7*	3.6*	3.1*	2.9*
		1.6	2.0	2.2*	2.7*	2.0*	1.2*
	W DMSO	.9	1.0	1.0	1.0s	.9s	1.0*
	dioxane	1.0	1.2	1.3	1.4s	1.5*	2.5*
	dioxane, manual						1.5*

* = ppt.
s = slight ppt.

Table 12

RO SAMPLES, FOUND MUTAGENIC BY SRI, REDONE BY W, AND HAND-COUNTED

TA 1538 (38), TA 98 (98) tester strain used.
1000 µg and 2500 µg hand counted

Controls: TA 1538: No act. 15; Act. 35
TA 98: No act. 32; Act. 31

R = Test count (ave. 3 plate) divided by respective control count.

	10 µg/plt		50 µg/plt		100 µg/plt		500 µg/plt		1000 µg/plt		2500 µg/plt	
RO #1	38	98	38	98	38	98	38	98	38	98	38	98
Counts												
1	11	36	14	38	16	37	28	59	32	51	29	81
2	12	37	7	26	11	32	13	38	17	34	20	51
3	8	28	5	34	9	46	11	31	10	44	13	53
5	10	22	14	30	17	28	23	28	24	32	38	33
9	8	23	11	19	7	31	11	25	7	22	9	24
12	10	27	8	35	7	46	6	21	8	20	5	17
15	8	19	8	22	8	23	10	19	9	17	9	20
16	8	22	11	31	9	30	19	26	22	24	13	33
20	10	19	13	18	14	20	11	12	10	13	11	17
22	9	18	12	14	9	20	10	17	10	16	11	16
23	10	16	9	16	9	18	10	16	15	12	10	13
R #												
1	.7	1.1	.9	1.2	1.1	1.2	1.9	1.8	2.1	1.6	1.9	2.5
2	.8	1.0	.5	.8	.7	1.0	.9	1.2	1.1	1.1	1.3	1.6
3	.5	.9	.3	1.1	.6	1.4	.7	1.0	.7	1.4	.9	1.7
5	.7	.7	.9	.9	1.1	.9	1.5	.9	1.6	1.0	2.5	1.0
9	.5	.7	.7	.6	.5	1.0	.7	.8	.5	.7	.6	.8
12	.7	.8	.5	1.1	.5	1.4	.4	.7	.5	.6	.3	.5
15	.5	.6	.5	.7	.5	.7	.7	.6	.6	.5	.6	.6
16	.5	.7	.7	1.0	.6	.9	1.3	.8	1.5	.8	.9	1.0
20	.7	.6	.9	.6	.9	.6	.7	.4	.7	.4	.7	.5
22	.6	.6	.8	.4	.6	.6	.7	.5	.7	.5	.7	.5
23	.7	.5	.6	.5	.6	.6	.7	.5	1.0	.4	.7	.4
With Enzyme Activation (59 Fraction)												
Counts												
1	35	41	43	42	52	66	135	126	119	122	156	131
2	28	30	27	44	32	36	55	57	64	66	79	96
3	36	36	24	47	26	30	32	48	35	48	37	59
5	31	40	37	43	45	61	87	120	87	91	114	110
9	26	40	22	39	24	45	22	44	16	26	17	31
12	33	41	36	53	29	55	41	60	25	36	38	38
15	31	36	18	42	24	46	27	59	31	59	37	48
16	31	50	35	61	42	79	91	120	99	123	147	121
20	30	33	23	38	18	42	27	50	22	40	25	51
22	28	32	20	27	19	42	30	58	37	60	41	73
23	26	44	23	45	18	57	50	80	47	46	47	73
R #												
1	1.0	1.3	1.2	1.4	1.5	2.1	3.9	4.1	3.4	3.9	4.5	4.2
2	.8	1.0	.8	1.4	.9	1.2	1.6	1.8	1.8	2.1	2.3	3.1
3	1.0	1.2	.7	1.5	.7	1.0	.9	1.5	1.0	1.5	1.1	1.9
5	.9	1.3	1.1	1.4	1.3	2.0	2.5	3.9	2.5	2.9	3.3	3.6
9	.7	1.3	.6	1.3	.7	1.5	.6	1.4	.5	.8	.5	1.0
12	.9	1.3	1.0	1.7	.8	1.8	1.2	1.9	.7	1.4	1.1	1.4
15	.9	1.2	.5	1.4	.7	1.5	.8	1.9	.9	1.9	1.1	1.6
16	.9	1.6	1.0	2.0	1.2	2.6	2.6	3.9	2.8	4.0	4.2	3.9
20	.9	1.1	.7	1.2	.5	1.4	.8	1.6	.6	1.3	.7	1.6
22	.8	1.0	.6	.9	.5	1.4	.9	1.9	1.1	1.9	1.2	2.4
23	.7	1.4	.7	1.5	.5	1.8	1.4	2.6	1.3	1.5	1.3	2.4

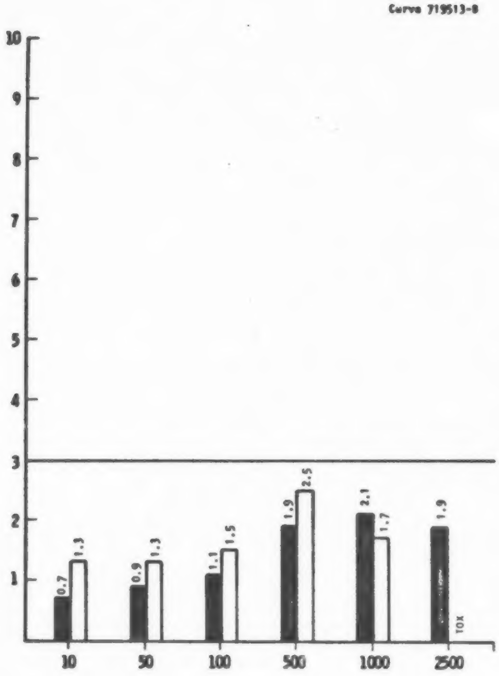

Fig. 2 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 1 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

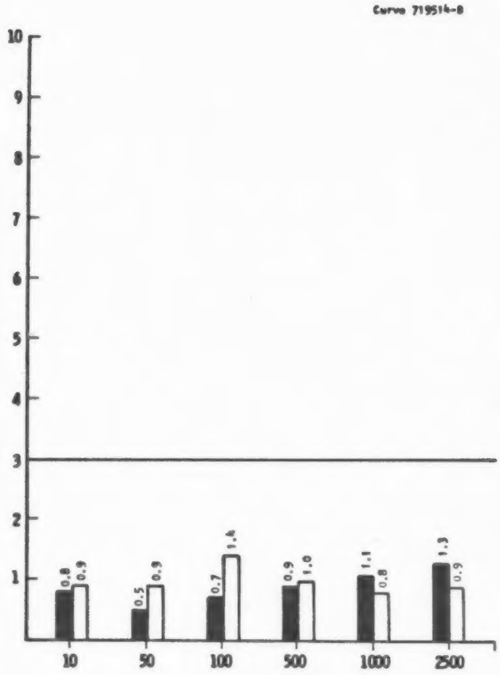

Fig. 3 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 2 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

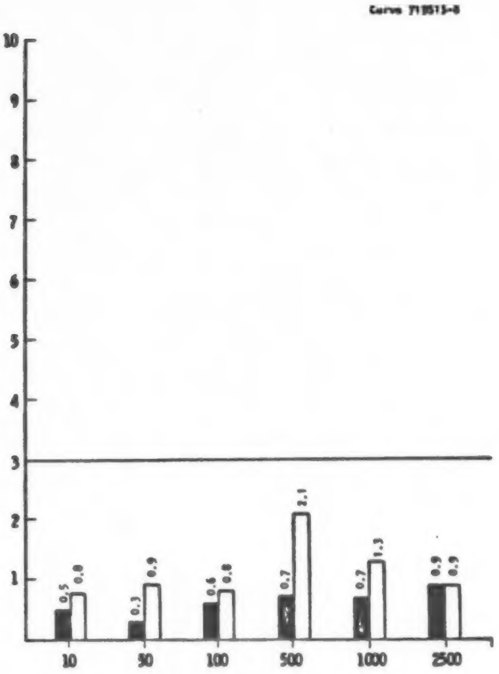

Fig. 4 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 3 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

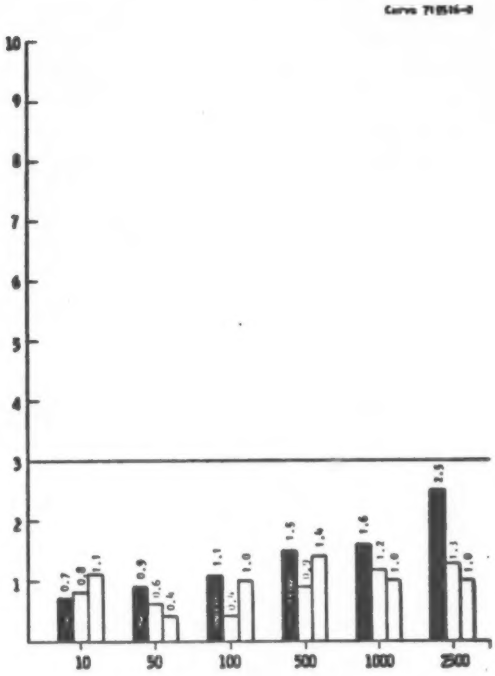

Fig. 5 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 5 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

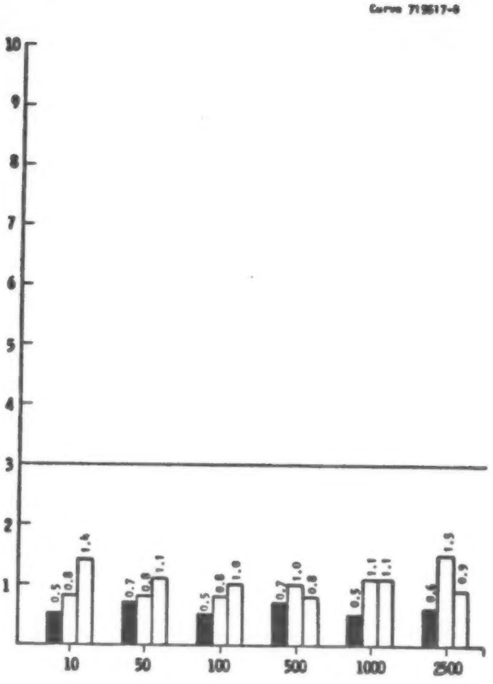

Fig. 6 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 9 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

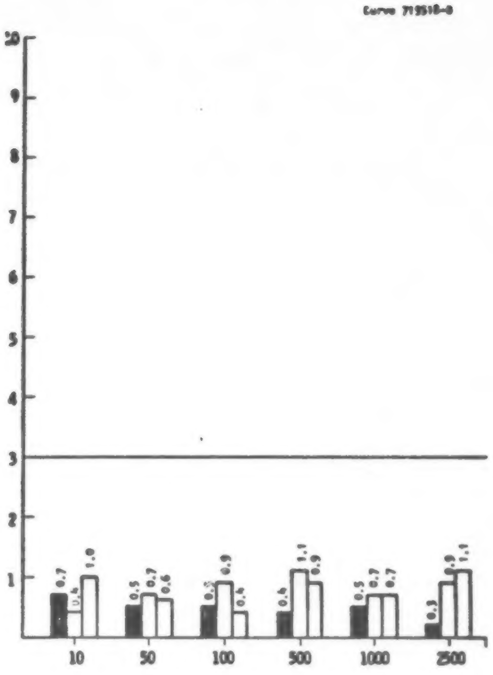

Fig. 7 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 12 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

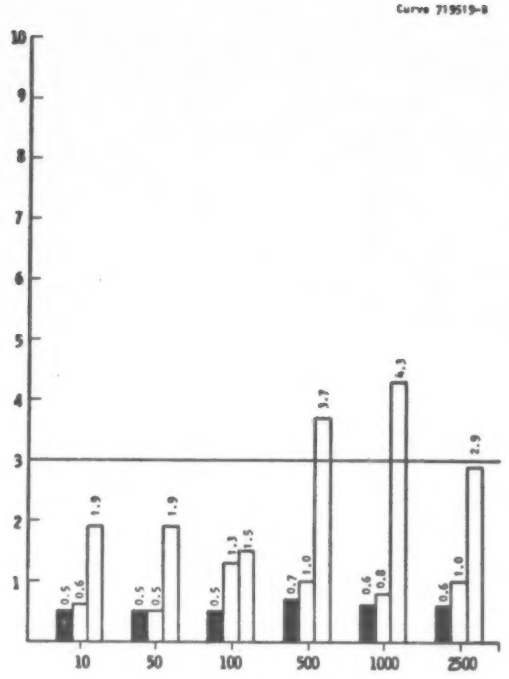

Fig. 8 — Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 15 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

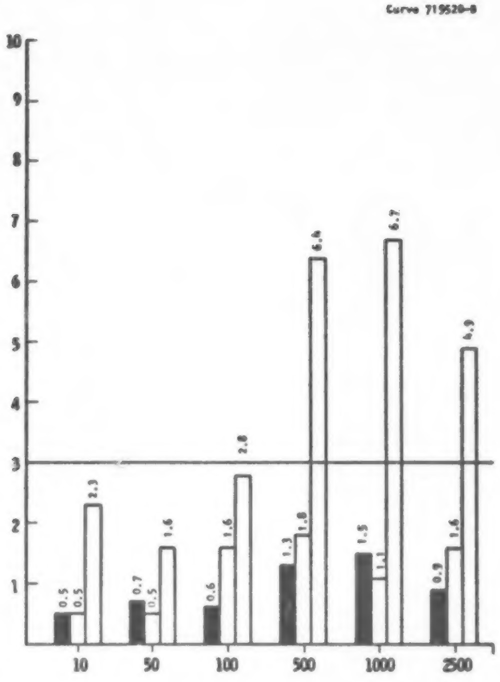

Fig. 9 — Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 16 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

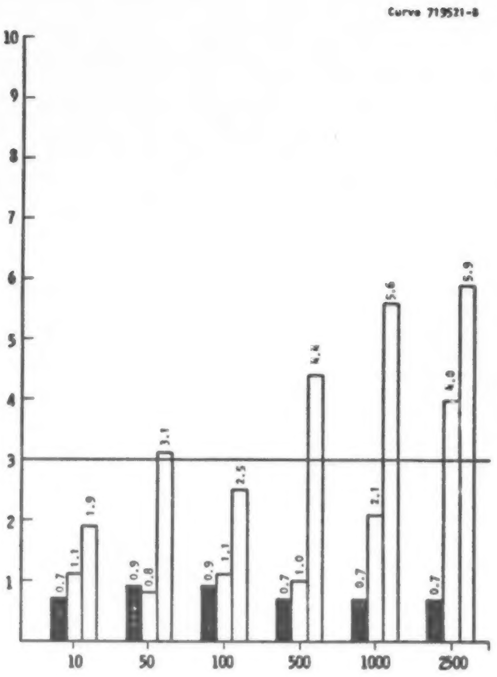

Fig. 10 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 20 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

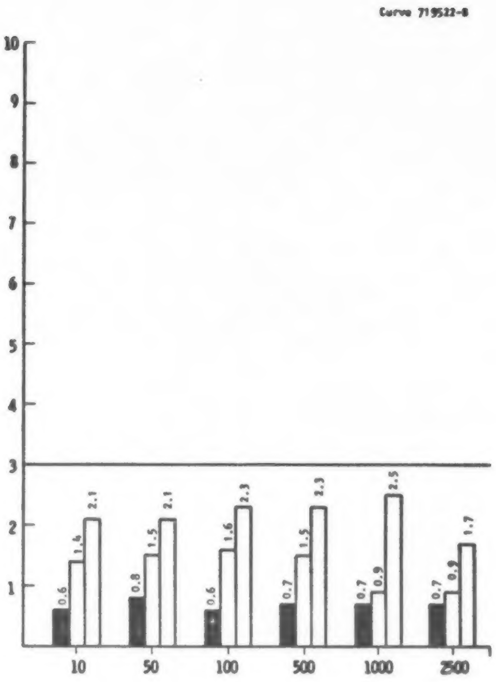

Fig. 11 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 22 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

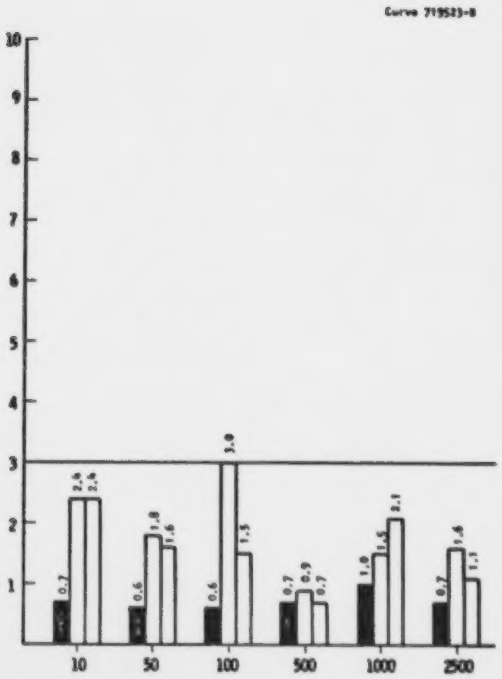

Fig. 12 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 23 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

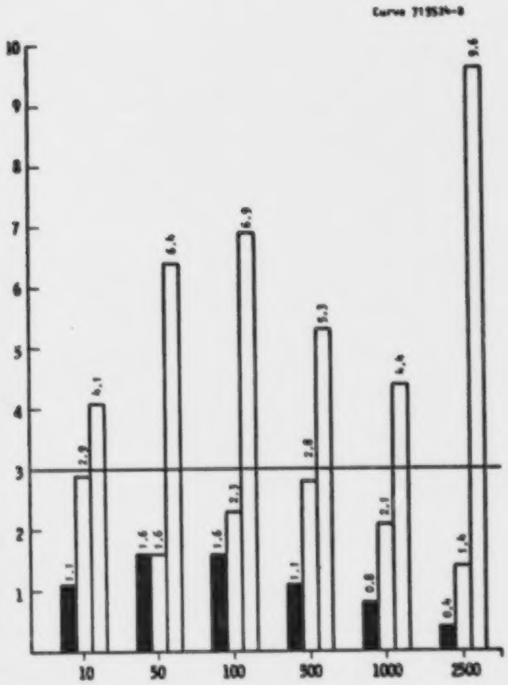

Fig. 13 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 24 Tester Strain TA 1538
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

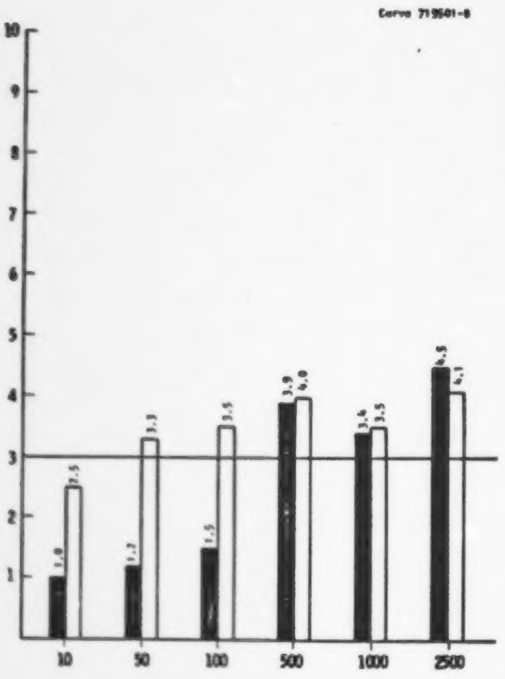

Fig. 14 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 1 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

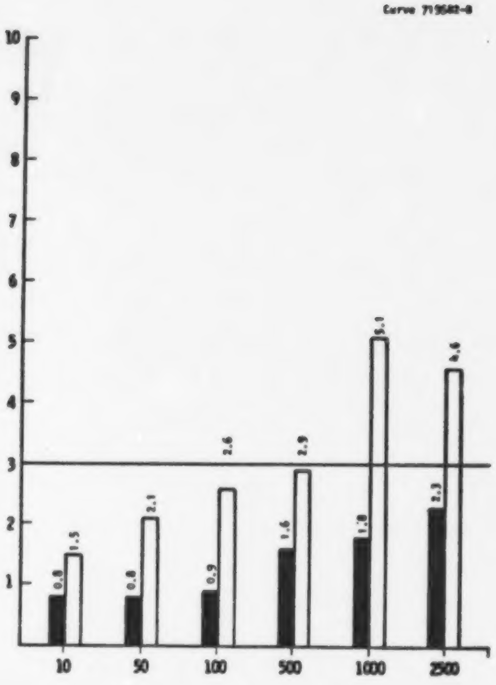

Fig. 15 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 2 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

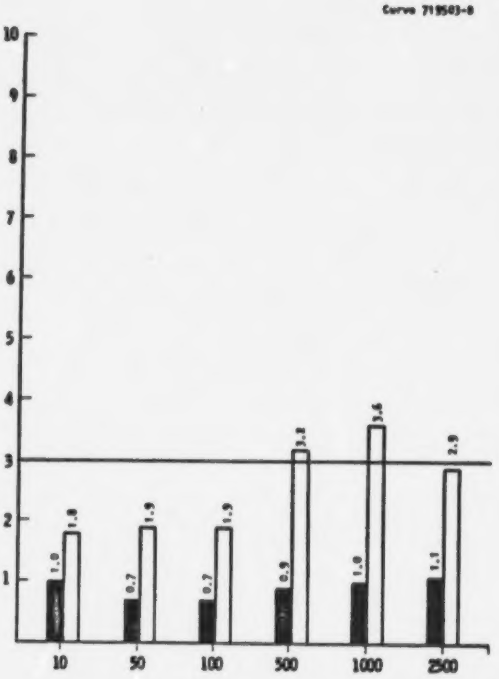

Fig. 16 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 3 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

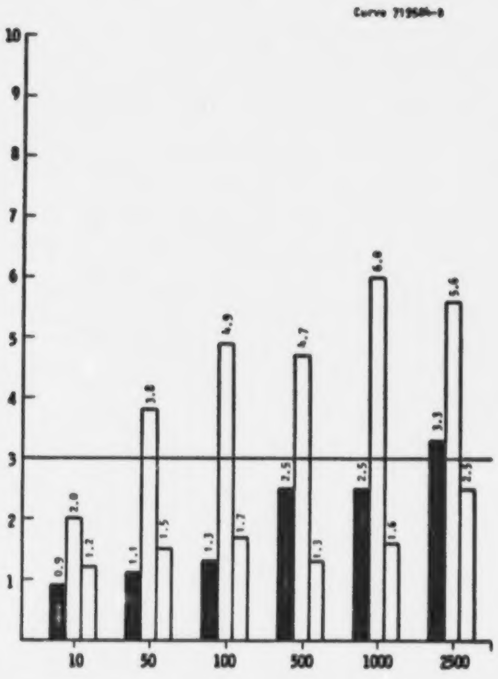

Fig. 17 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 5 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

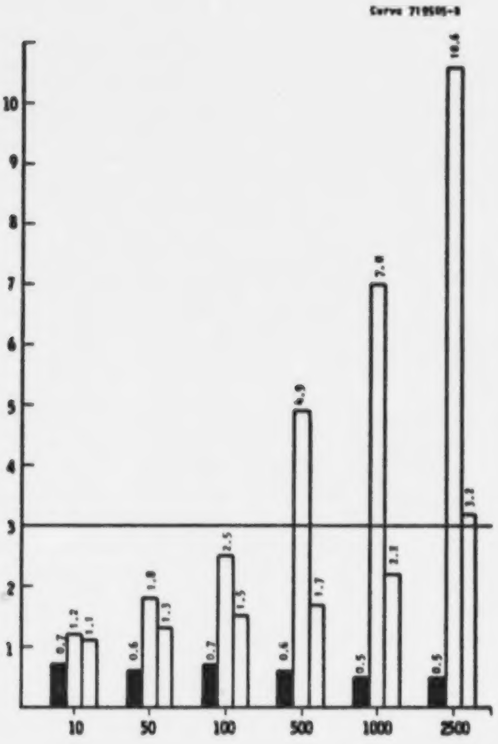

Fig. 18 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 9 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

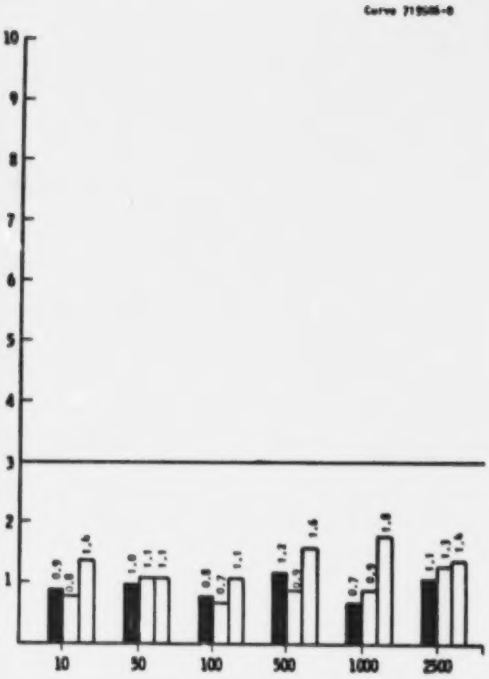

Fig. 19 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 12 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

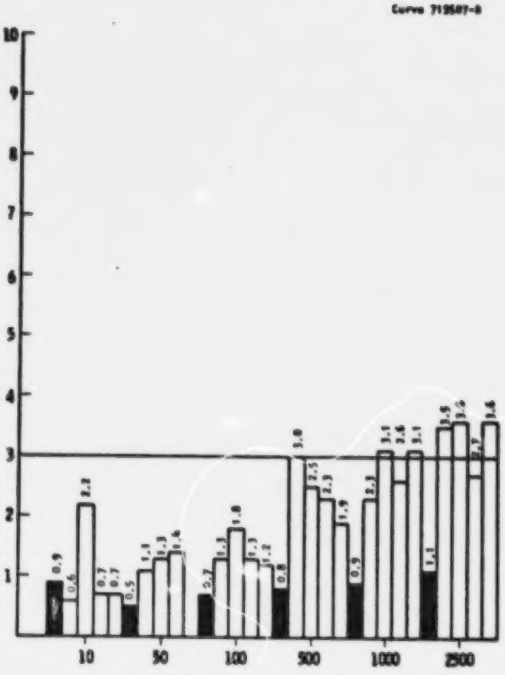

Fig. 20 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 15 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

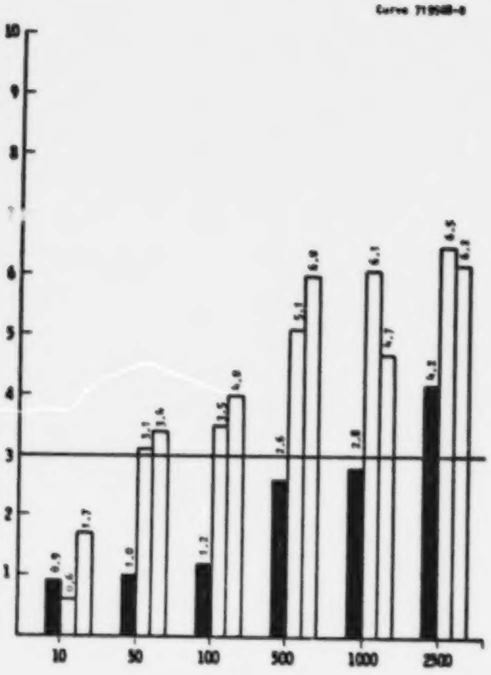

Fig. 21 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 16 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

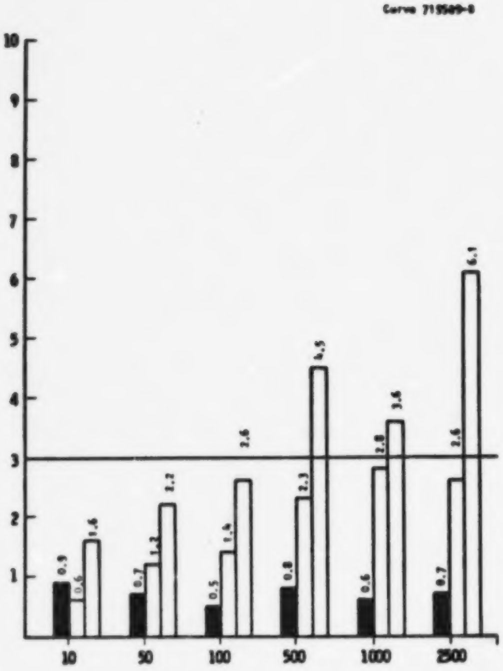

Fig. 22 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 20 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

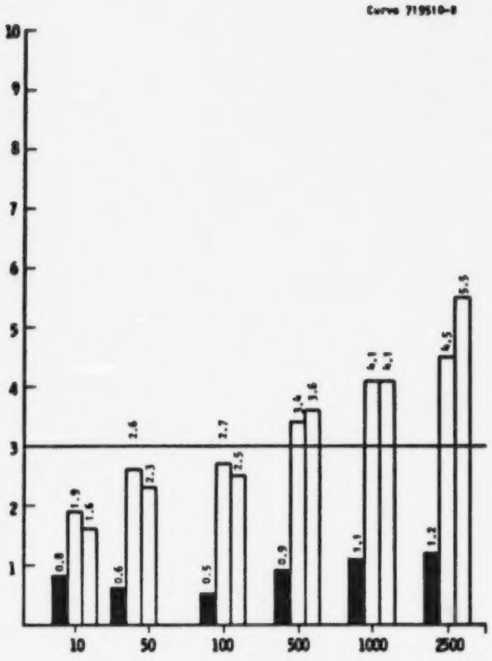

Fig. 23 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 22 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

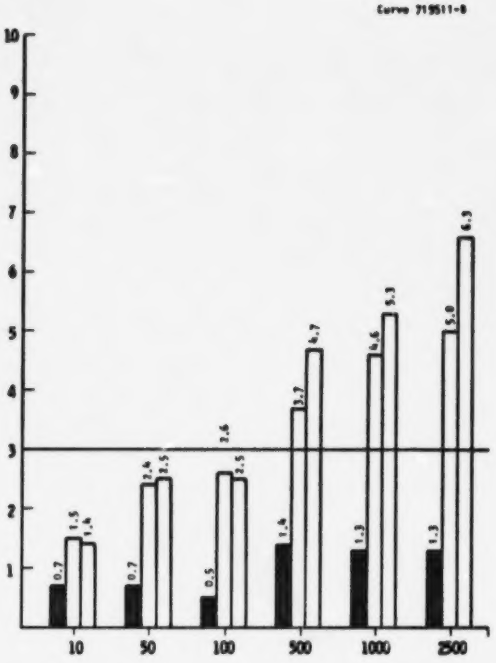

Fig. 24 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 23 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

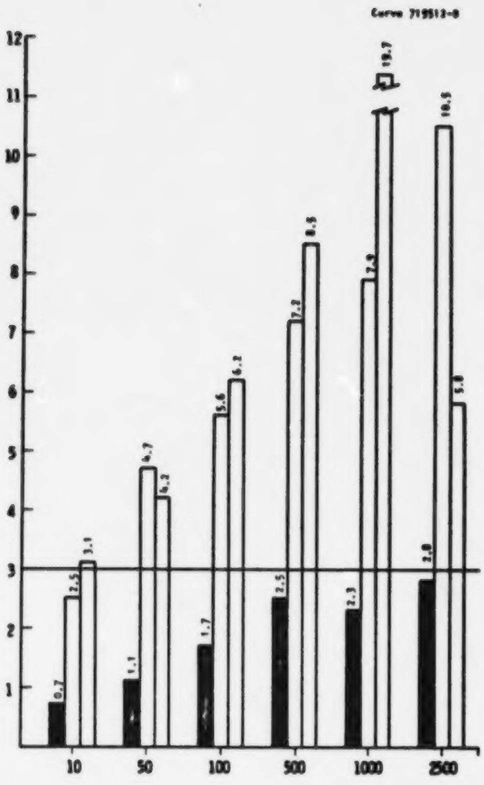

Fig. 25 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 24 Tester Strain TA 1538
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

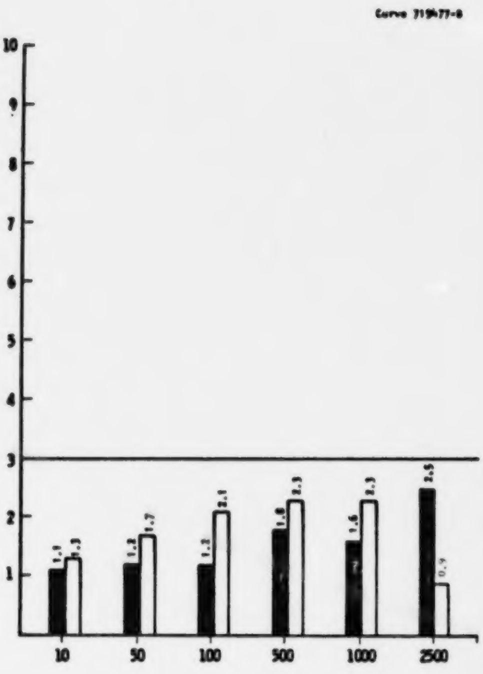

Fig. 26 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 1 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

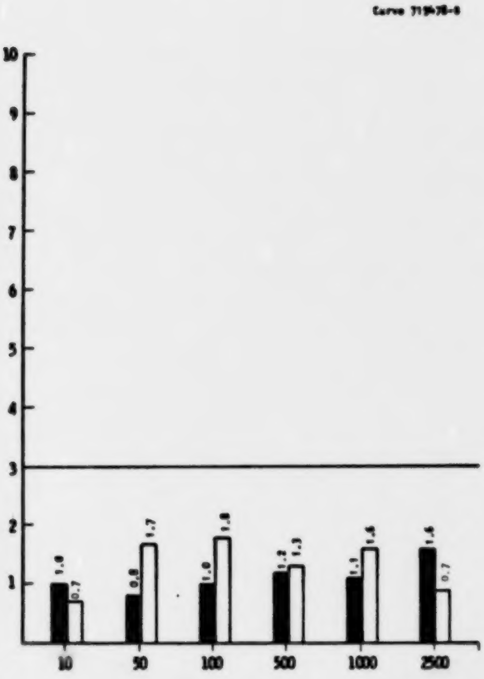

Fig. 27 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 2 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

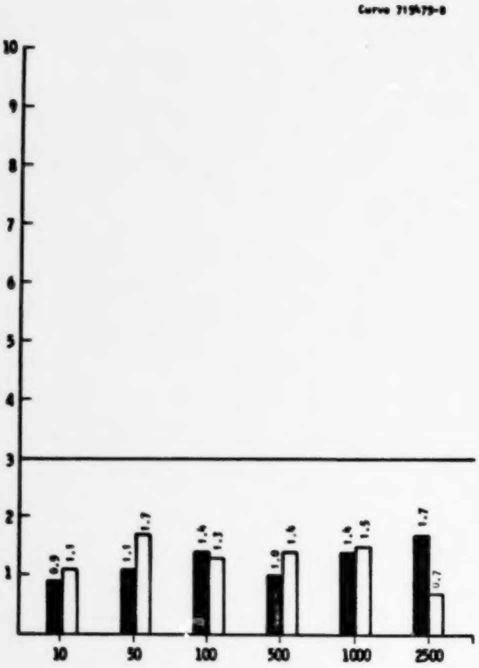

Fig. 28 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 3 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

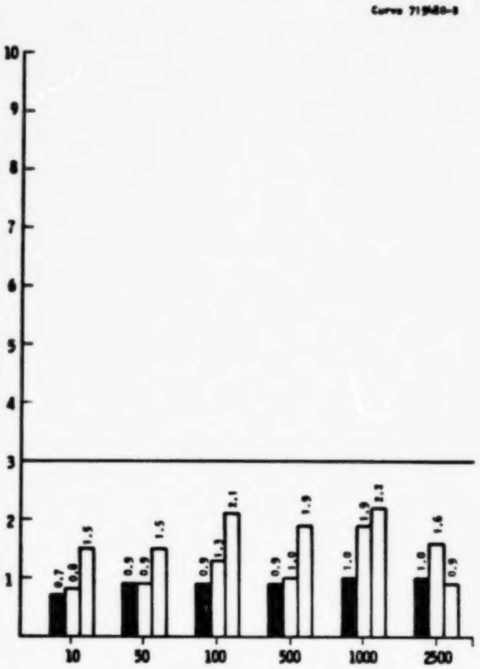

Fig. 29 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 5 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

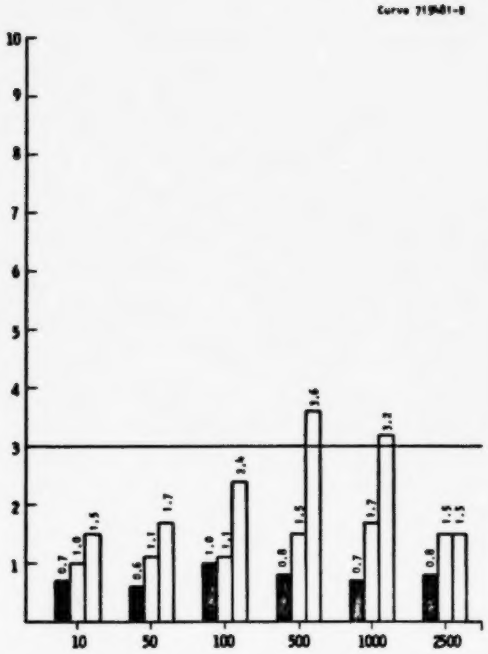

Fig. 30 — Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 9 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

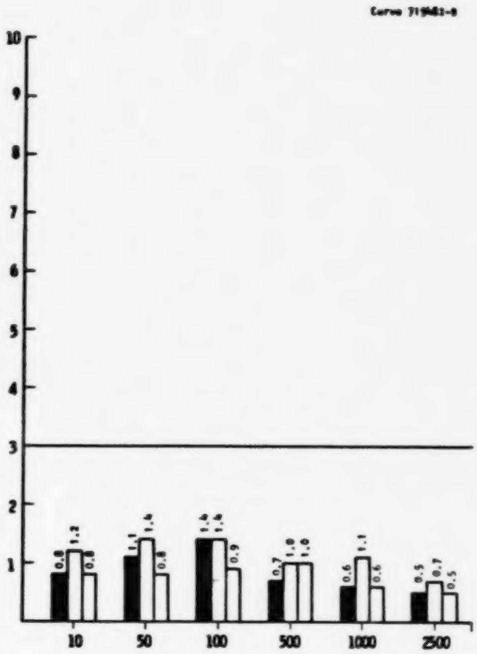

Fig. 31 — Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 12 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

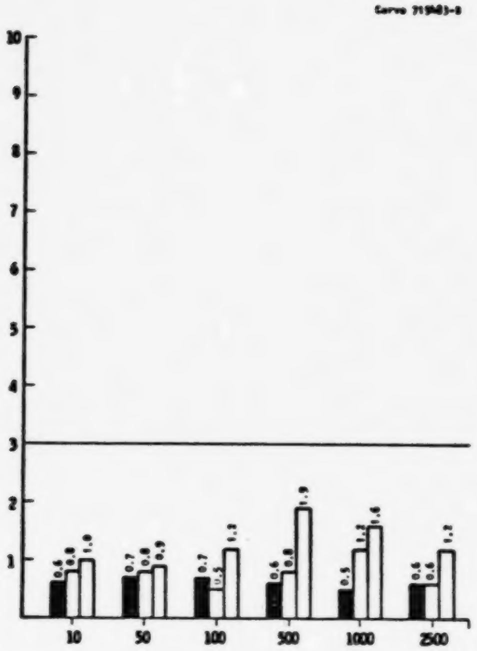

Fig. 32 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 15 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

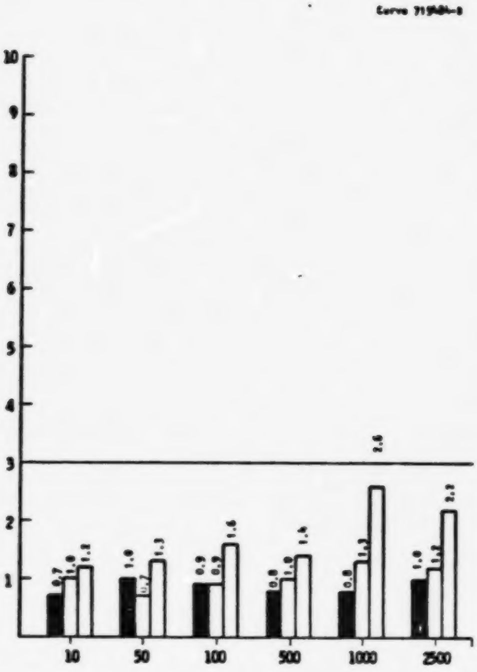

Fig. 33 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 16 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

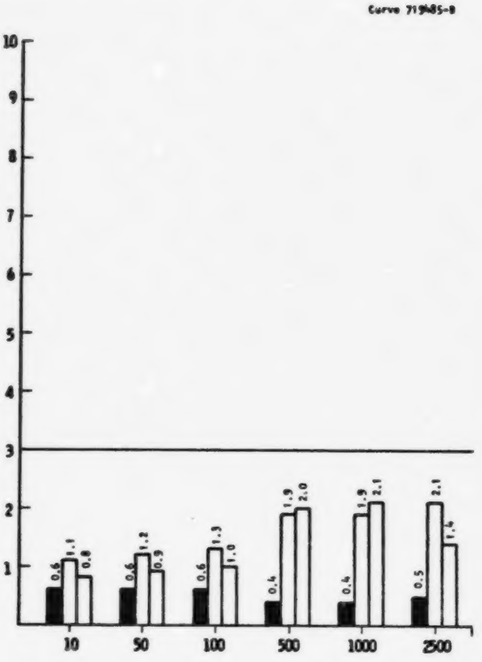

Fig. 34 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 20 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

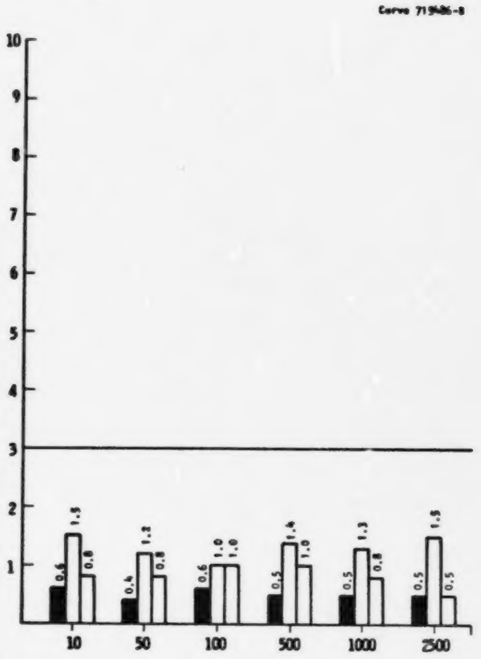

Fig. 35 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 22 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

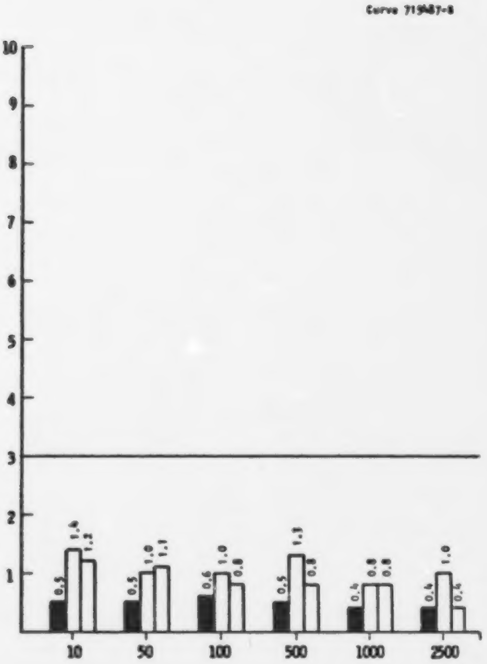

Fig. 36 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 23 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

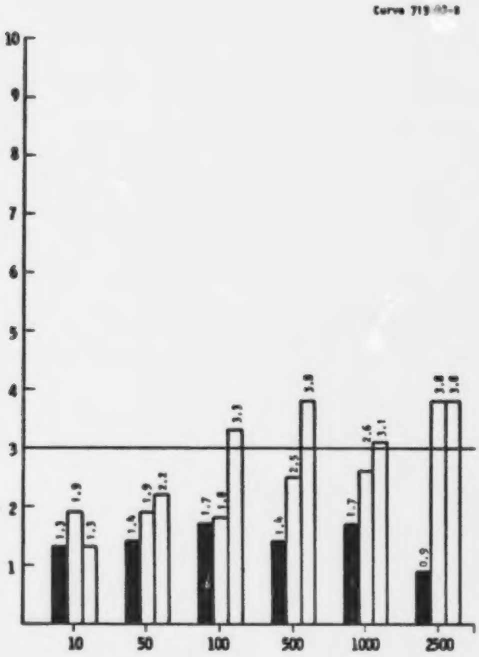

Fig. 37 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 24 Tester Strain TA 98
Microsome Activation (S9) No

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

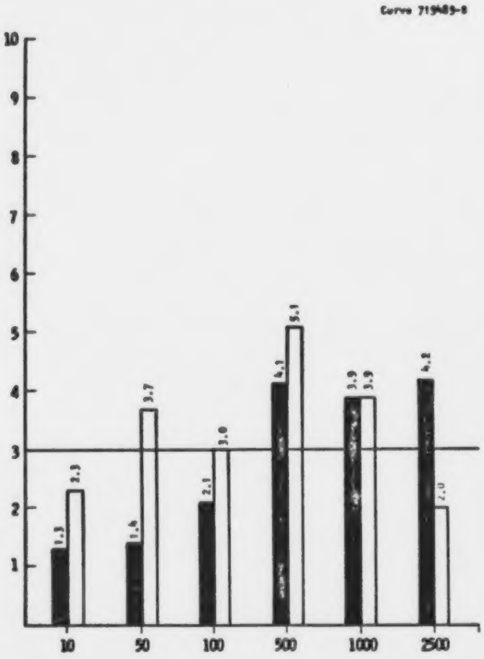

Fig. 38 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 1 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

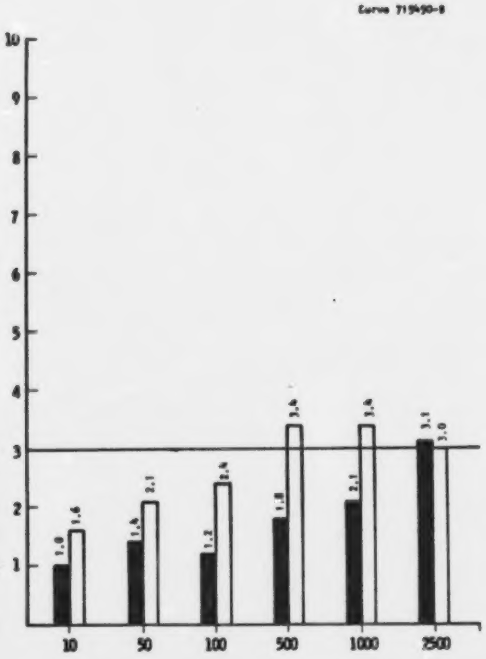

Fig. 39 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 2 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

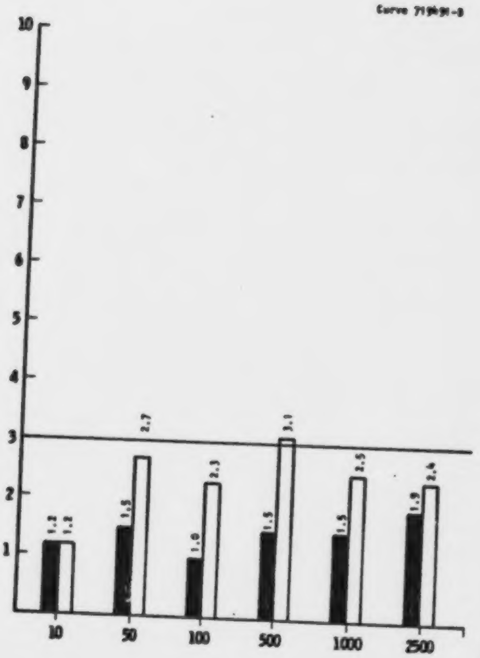

Fig. 40 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 3 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

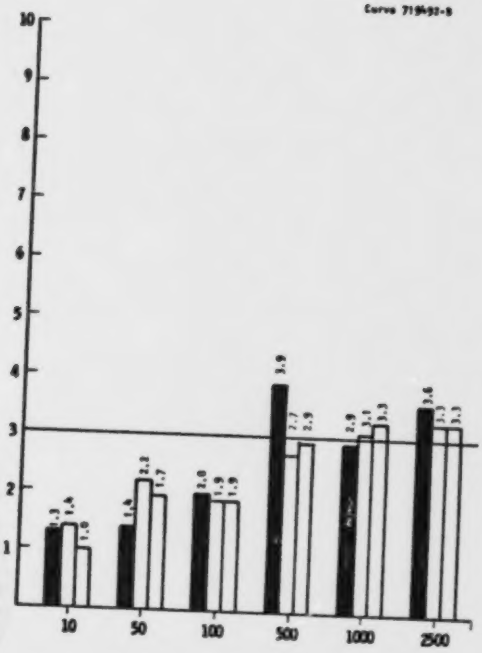

Fig. 41 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 5 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

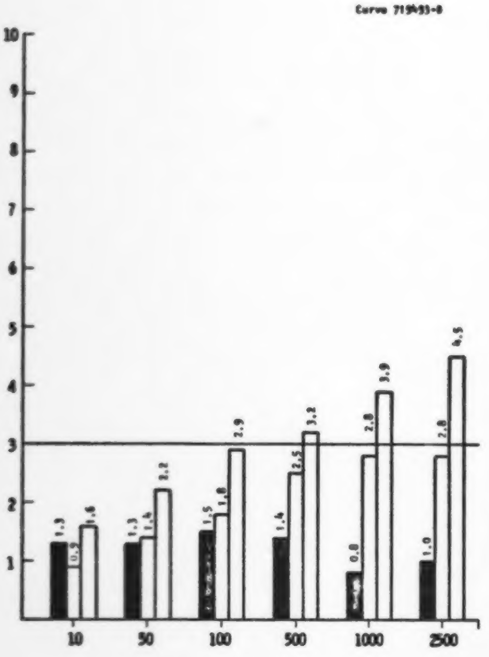

Fig. 42 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 9 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

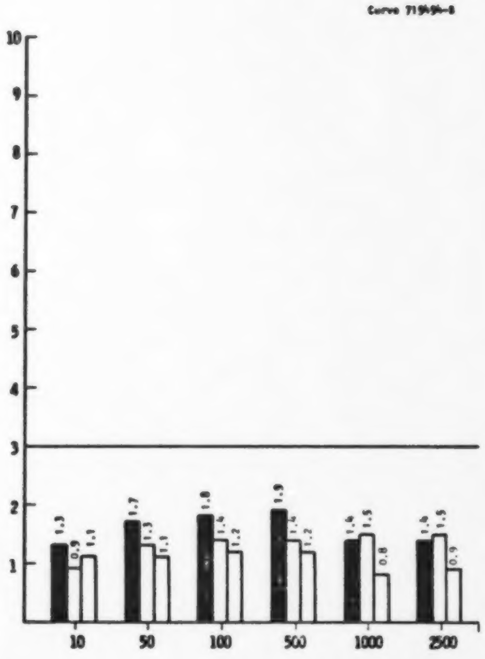

Fig. 43 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 12 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

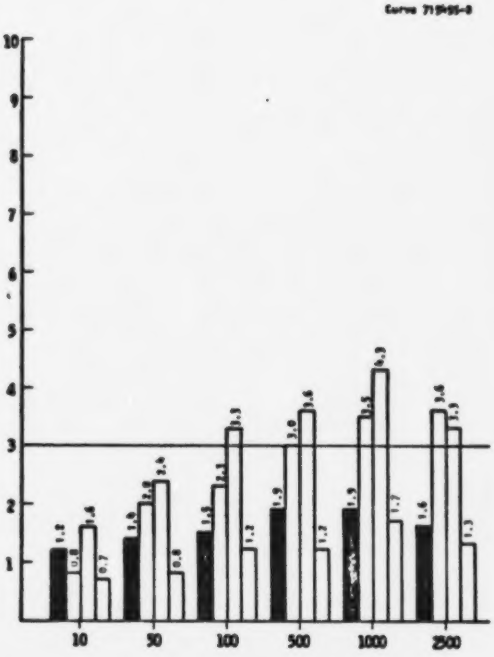

Fig. 44 — Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 15 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

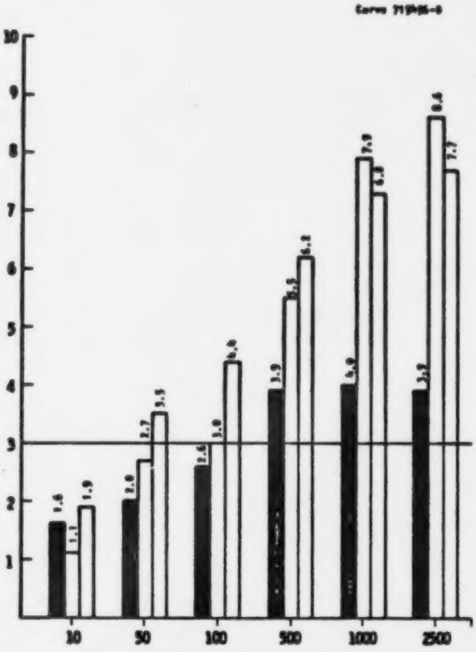

Fig. 45 — Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 16 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

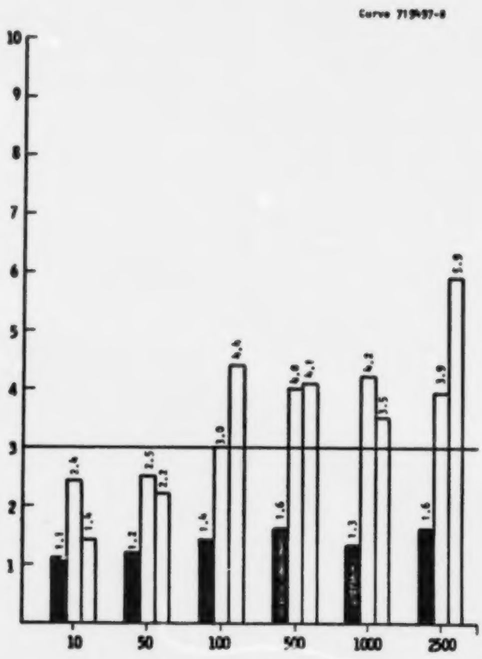

Fig. 46 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 20 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

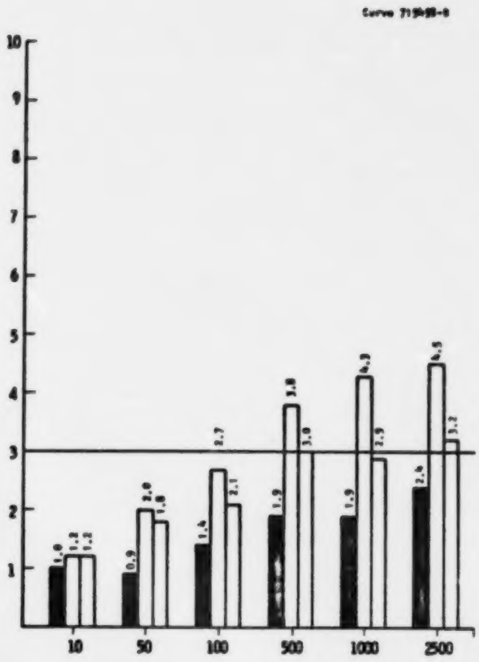

Fig. 47 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 22 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

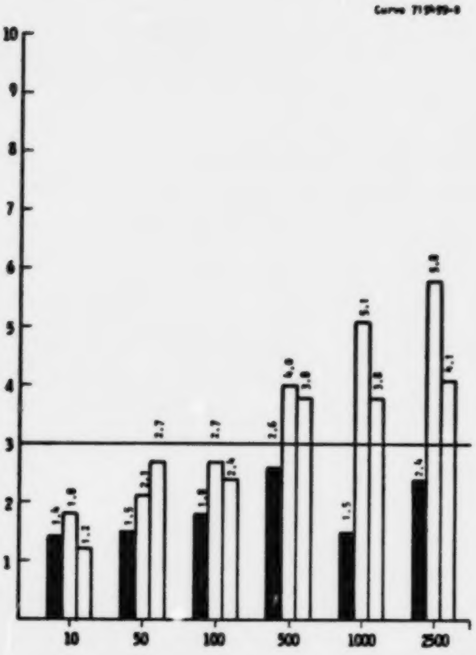

Fig. 48 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 23 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

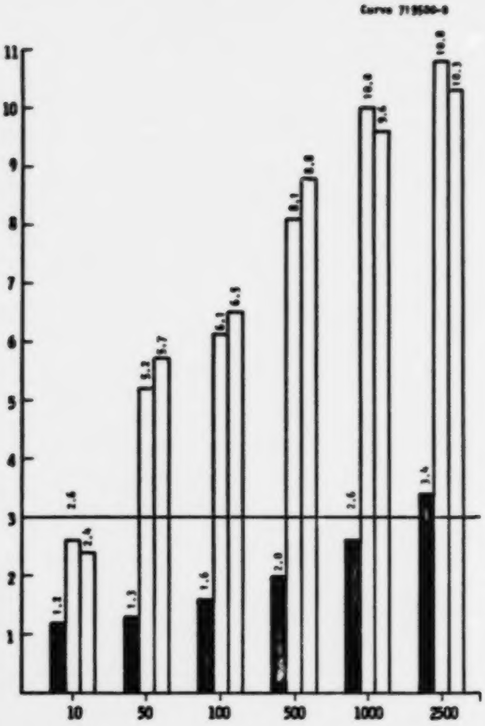

Fig. 49 – Comparison Ⓦ and SRI AMES test data of positive samples

Sample RO 24 Tester Strain TA 98
Microsome Activation (S9) Yes

For direct comparison the results are expressed as R ratio (Test over control revertant counts). A ratio of ≧ 3 is considered positive response. Solvent used is p-dioxane. Each bar represents one test. Shaded bar is Ⓦ test. X-axis is test chemical per plate in micrograms. Y-axis is R ratio value.

Table 13

COMPARISON OF W AND SRI AMES TEST DATA OF POSITIVE SAMPLES USING TA 1538

For direct comparison the results are expressed as the ratio of test to control revertants. A ratio of 3 or higher is considered a positive response. The solvent used is p-dioxane. Sample RO #12 is negative in both W and SRI test and is included as control.

Quantity of oil sample in micrograms per plate

		10			50			100			500			1000			2500		
	RO#	W		SRI	W		SRI	W		SRI	W		SRI	W		SRI	W		SRI
NO ACTIVATION	1	.7		1.3	.9		1.3	1.1		1.5	1.9		2.5	2.1		1.7	1.9		Tox
	2	.8		.9	.5		.9	.7		1.4	.9		1.0	1.1		.8	1.3		.9
	3	.5		.8	.3		.9	.6		.8	.7		2.1	.7		1.3	.9		.9
	5	.7	.8	1.1	.9	.6	.4	1.1	.4	1.0	1.5	.9	1.4	1.6	1.2	1.0	2.5	1.3	.9
	9	.5	.8	1.4	.7	.8	1.1	.5	.8	1.0	.7	1.0	.8	.5	1.1	1.1	.6	1.5	.9
	12	.7	.4	1.0	.5	.7	.6	.5	.9	.4	.4	1.1	.9	.5	.7	.7	.3	.9	1.1
	15	.5	.6	1.9	.5	.5	1.9	.5	1.3	1.5	.7	1.0	3.7	.6	.8	4.3	.6	1.0	2.9
	16	.5	.5	2.3	.7	.5	1.6	.6	1.6	2.8	1.3	1.8	6.4	1.5	1.1	6.7	.9	1.6	4.9
	20	.7	1.1	1.9	.9	.8	3.1	.9	1.1	2.5	.7	1.0	4.4	.7	2.1	5.6	.7	4.0	5.9
	22	.6	1.4	2.1	.8	1.5	2.1	.6	1.6	2.3	.7	1.5	2.3	.7	.9	2.5	.7	.9	1.7
	23	.7	2.4	2.4	.6	1.8	1.6	.6	3.0	1.5	.7	.9	.7	1.0	1.5	2.1	.7	1.6	1.1
	24	1.1	2.9	4.1	1.6	1.6	6.4	1.6	2.3	6.9	1.1	2.8	5.3	.8	2.1	4.4	.4	1.4	9.6
MICROSOME ACTIVATION (S9)	1	1.0		2.5	1.2		3.3	1.5		3.5	3.9		4.0	3.4		3.5	4.5		4.1
	2	.8		1.5	.8		2.1	.9		2.6	1.6		2.9	1.8		5.1	2.3		4.6
	3	1.0		1.8	.7		1.9	.7		1.9	.9		3.2	1.0		3.6	1.1		2.9
	5	.9	2.0	1.2	1.1	3.8	1.5	1.3	4.9	1.7	2.5	4.7	1.3	2.5	6.0	1.6	3.3	5.6	2.5
	9	.7	1.2	1.1	.6	1.8	1.3	.7	2.5	1.5	.6	4.9	1.7	.5	7.0	2.2	.5	10.6	3.2
	12	.9	.8	1.4	1.0	1.1	1.1	.8	.7	1.1	1.2	.9	1.6	.7	.9	1.8	1.1	1.3	1.4
	15a	.9	.6	.7	.5	1.1	1.3	.7	1.3	1.3	.8	3.0	2.3	.9	2.3	2.6	1.1	3.5	2.7
	15b		2.2	.7			1.4		1.8	1.2		2.5	1.9		3.1	3.1		3.6	3.6
	16	.9	.6	1.7	1.0	3.1	3.4	1.2	3.5	4.0	2.6	5.1	6.0	2.8	6.1	4.7	4.2	6.5	6.2
	20	.9	.6	1.6	.7	1.2	2.2	.5	1.4	2.6	.8	2.3	4.5	.6	2.8	3.6	.7	2.6	6.1
	22	.8	1.9	1.6	.6	2.6	2.3	.5	2.7	2.5	.9	3.4	3.6	1.1	4.1	4.1	1.2	4.5	5.5
	23	.7	1.5	1.4	.7	2.4	2.5	.5	2.6	2.5	1.4	3.7	4.7	1.3	4.6	5.3	1.3	5.0	6.6
	24	.7	2.5	3.1	1.1	4.7	4.2	1.7	5.6	6.2	2.5	7.2	8.5	2.3	7.9	19.7	2.8	10.5	5.8

Table 14

COMPARISON OF W AND SRI AMES TEST DATA OF POSITIVE SAMPLES USING TA 98

For direct comparison the results are expressed as the ratio of test to control revertants. A ratio of 3 or higher is considered a positive response. The solvent used is p-dioxane. Sample RO #12 is negative in both W and SRI test and is included as control.

		Quantity of oil sample in micrograms per plate																	
		10			50			100			500			1000			2500		
	RO#	W	SRI		W	SRI		W	SRI		W	SRI		W	SRI		W	SRI	
NO ACTIVATION	1	1.1	1.3		1.2	1.7		1.2	2.1		1.8	2.3		1.6	2.3		2.5	.9	
	2	1.0	.7		.8	1.7		1.0	1.8		1.2	1.3		1.1	1.6		1.6	.7	
	3	.9	1.1		1.1	1.7		1.4	1.3		1.0	1.4		1.4	1.5		1.7	.7	
	5	.7	.8	1.5	.9	.9	1.5	.9	1.3	2.1	.9	1.0	1.9	1.0	1.9	2.2	1.0	1.6	.9
	9	.7	1.0	1.5	.6	1.1	1.7	1.0	1.1	2.4	.8	1.5	3.6	.7	1.7	3.2	.8	1.5	1.5
	12	.8	1.2	.8	1.1	1.4	.8	1.4	1.4	.9	.7	1.0	1.0	.6	1.1	.6	.5	.7	.5
	15	.6	.8	1.0	.7	.8	.9	.7	.5	1.2	.6	.8	1.9	.5	1.2	1.6	.6	.6	1.2
	16	.7	1.0	1.2	1.0	.7	1.3	.9	.9	1.6	.8	1.0	1.4	.8	1.3	2.6	1.0	1.2	2.2
	20	.6	1.1	.8	.6	1.2	.9	.6	1.3	1.0	.4	1.9	2.0	.4	1.9	2.1	.5	2.1	1.4
	22	.6	1.5	.8	.4	1.2	.8	.6	1.0	1.0	.5	1.4	1.0	.5	1.3	.8	.5	1.5	.5
	23	.5	1.4	1.2	.5	1.0	1.1	.6	1.0	.8	.5	1.3	.8	.4	.8	.8	.4	1.0	.4
	24	1.3	1.9	1.3	1.4	1.9	2.2	1.7	1.8	3.3	1.4	2.5	3.8	1.7	2.6	3.1	.9	3.8	3.8
MICROSOME ACTIVATION (S9)	1	1.3	2.3		1.4	3.7		2.1	3.0		4.1	5.1		3.9	3.9		4.2	2.0	
	2	1.0	1.6		1.4	2.1		1.2	2.4		1.8	3.4		2.1	3.4		3.1	3.0	
	3	1.2	1.2		1.5	2.7		1.0	2.3		1.5	3.1		1.5	2.5		1.9	2.4	
	5	1.3	1.4	1.0	1.4	2.2	1.7	2.0	1.9	1.9	3.9	2.7	2.9	2.9	3.1	3.3	3.6	3.3	3.3
	9	1.3	.9	1.6	1.3	1.4	2.2	1.5	1.8	2.9	1.4	2.5	3.2	.8	2.8	3.9	1.0	2.8	4.5
	12	1.3	.9	1.1	1.7	1.3	1.1	1.8	1.4	1.2	1.9	1.4	1.2	1.4	1.5	.8	1.4	1.5	.9
	15a	1.2	.8	1.6	1.4	2.0	2.4	1.5	2.3	3.3	1.9	3.0	3.6	1.9	3.5	4.3	1.6	3.6	3.3
	15b		.7			.8			1.2			1.2			1.7			1.3	
	16	1.6	1.1	1.9	2.0	2.7	3.5	2.6	3.0	4.4	3.9	5.5	6.2	4.0	7.9	6.8	3.9	8.6	7.7
	20	1.1	2.4	1.4	1.2	2.5	2.2	1.4	3.0	4.4	1.6	4.0	4.1	1.3	4.2	3.5	1.6	3.9	5.9
	22	1.0	1.2	1.2	.9	2.0	1.8	1.4	2.7	2.1	1.9	3.8	3.0	1.9	4.3	2.9	2.4	4.5	3.2
	23	1.4	1.8	1.2	1.5	2.1	2.7	1.8	2.7	2.4	2.6	4.0	3.8	1.5	5.1	3.8	2.4	5.8	4.1
	24	1.2	2.6	2.4	1.3	5.2	5.7	1.6	6.1	6.5	2.0	8.1	8.8	2.6	10.0	9.6	3.4	10.8	10.3

From the results we observe that RO #12 was negative with good W and SRI data correlation. In the other samples, however, differences are present. Each strain will be separately discussed.

TA 1538 - No Activation

All W samples were negative for mutagenicity and seven SRI were alike in response. SRI RO #15 must be classified as negative. The W and first SRI run have good correlation, but the second test, done ten days later, shows a positive response. The average of the two tests, however, is less than double increase over the control, and the difference between the two SRI tests is considerable.

Two SRI samples, RO #16 and RO #24, are negative in the first run but strongly positive in the second, done ten days later. RO #16, is very similar to RO #15 in correlation with W on first test and divergence between test one and two. Due to the strong response on the second test, the average exceeds double increase over the control. RO #24 correlates somewhat less well with W test, but the internal variation is especially great when the highest concentration is analyzed.

TA 1538 - Metabolic Activation

From the SRI data nine tests have clear cut positive response. RO #5 and RO #20 are positive in only one out of two tests. Westinghouse data has only three positives (RO #1, #5, #16) with RO #24 showing a dose response increase, however, not to a full double increase over the control. RO #5, positive in W, is negative in the second SRI test. The activity is, nevertheless, judged positive, because W and SRI are positive. RO #20 should be considered mutagenic because the first SRI test, although, negative has a dose response curve and is close to a double control increase. The W R ratios are low without any correlation to concentration. It must also be pointed out that RO #15 was tested four times by SRI, the test being all negative.

TA 98 - No Activation

Without activation all Westinghouse tests were negative. RO #24 is positive in SRI tests. RO #9 in SRI test one is negative, while the second one, run 12 days later, is positive. The average of both tests being less than R ratio of 3, the sample is judged negative. Thus, except for RO #24 there is a good agreement between the results of SRI and W.

TA 98 - Metabolic Activation

With the addition of S9 MIX, five Westinghouse samples were positive, while 10 of SRI were found to be mutagenic. RO #3 presents a questionable response in SRI data. Only one test was performed and, although at 500 μg per plate a barely positive response was elicited, there is no dose response curve, hence mutagenicity is either very weak or else not present at all. It should also be noted that the two SRI tests on RO #9 were first negative, but with a dose response curve, and in the second round of tests positive. The overall reaction is one of mutagenicity. The RO #15 sample was tested three times. The results demonstrate well the variations observed. Westinghouse results are negative with a relatively level response. The first and second SRI tests, ten days apart are positive with a good dose response. The third SRI test, done 35 days later is negative, with a low and even response, which is slightly lower than the W results.

When these sample results of the eleven in TA 1538 and TA 98 with activation positive SRI and one negative oil residue are compared with the W test results under same test conditions, a discrepancy in mutagenicity becomes apparent. Westinghouse data shows mutagenicity in only three TA 1538 and five TA 98 samples with enzyme activation. The cause for this is not known, but several hypothesis and general observations will be presented.

Observations

In the negative sample RO #12 there is a good correlation. In the non-activated samples which are not mutagenic, there is a good correlation, although there is a tendency for the SRI results to be somewhat higher. Without activation in both strains of dual SRI results, the first in negative, the second positive. With activation on the other hand there is no pattern between the two test periods. The only interesting case is RO #15 in TA 98 were the third test, performed 35 days later is negative without a dose response.

The genetic quality of the tester strains cannot be faulted, because both laboratories did a regular genetic marker check. The general tester strain response is probably also not the cause, because the positive controls done with each test run, have performed well. Although there is considerable variation in the magnitude of response between individual tests, a positive test reaction does not appear to correlate with a high positive control response. The overall performance and quality of testing, by following a well defined procedure, did not differ between the laboratories. This is well demonstrated by the agreement in all negative samples. The disagreement in the results is only present in samples which exhibit a mutagenic response. At this time the only reasonable variable which remains is the age of the p-dioxane solubilized sample. At Westinghouse a sample is solubilized and tested within a week. At SRI the first sample was tested 17 or 18 days after preparation, the second usually 10 days (27 or 28 days) later. Only when a third test was done, the time span increased, with activation to 42 days in RO #15 TA 1538 and to 65 days on TA 98 with activation. In both cases the results were negative, in the third test, however, on TA 1538 a fourth test on the 63rd day was positive.

It is not clearly demonstrated that age of the dissolved sample has an effect on its mutagenic response, but it seems plausible that this might be the case with samples which are either weakly mutagenic or have very small amounts of mutagenic substances present in their composition.

Column Chromatography Pre-Separation Fractions

Our column chromatography pre-separation fractions were subjected to bioassay. Six oils were selected, fractionated into seven parts and analyzed biologically using the Ames test by SRI. The *E. coli* and yeast D4 tests were omitted, as it had been shown, that negative results are to be expected. The six oils selected were RO #1, 5, 12, 14, 15, and 16. The previous discussions of the bioassays of the 26 oils were the basis for fractionation. RO #12 and RO #14 were both found to be non-mutagenic, while the others were all frameshift mutagens.

To facilitate the discussion of the results, the data is presented in bar graph form from Figure 1 to Figure 168 in Appendix C. The summary of results is presented in Table 15 to Table 20. The details of the SRI data analysis are in Appendix B. Table 21 presents the data as plus (+), questionable (Q) and positive (P) for two test average. Plus means that a positive response was present in the test with a dose-response indication for the dose levels 10, 50, 100, 500, 1000 and 2500 μg of respective oil fraction per plate. Empty space means that there was a negative response, except for fraction #7, which, because of small quantity of material, was only tested once. Questionable response is one where the R was equal to or exceeded the value of three (3) without any dose-response indication. When the average of R values for both tests equaled or exceeded three (3), that set is judged positive (P). Table 22 presents all P values as pluses for ease of analysis. From these tables several conclusions can be drawn.

As in the preceding evaluations, two things become immediately apparent. First is that of two assayed fractions, in some cases only one is mutagenic. The second aspect is the extremely conservative Stanford Research International Inc. evaluation of weak mutagens, resulting in more positives than we judge based on the criteria previously outlined on page 59 . The first part of the discussion of the results will revolve around a re-evaluation of SRI conclusions. There is complete

Table 15

SR1 AMES TEST DATA ON RESIDUAL OIL FRACTIONS

The numerical data is presented as R ratio values (test over control revertants)

Each fraction, except #7, was tested at two separate times, hence two values for each concentration.

OIL RO NUMBER	TESTER STRAIN	FRACTION	NO ACTIVATION												MICROSOME ACTIVATION											
			Quantity of Sample in micrograms per plate												Quantity of Sample in micrograms per plate											
			10		50		100		500		1000		2500		10		50		100		500		1000		2500	
1	1537	1	0.6	1.4	0.5	1.2	1.0	1.8	1.0	1.2	0.6	1.4	0.3	0.7	1.3	0.9	0.8	0.9	1.5	1.1	1.0	0.9	0.7	2.0	1.5	1.2
		2	0.7	0.9	1.0	1.0	2.5	2.1	0.4	1.7	0.4	1.4	1.4	1.5	1.1	1.7	0.8	1.6	2.3	2.0	1.8	1.7	2.1	1.9	1.7	1.3
		3	1.3	1.3	1.9	1.6	1.9	1.7	1.1	3.3	1.1	2.9	0.4	1.8	0.5	1.9	0.8	2.2	1.0	2.9	1.0	3.1	1.2	3.3	1.0	3.3
		4	0.5	1.4	0.9	1.4	1.2	2.1	1.7	1.9	2.1	1.9	2.0	---	0.4	1.5	0.5	1.6	0.5	1.8	0.7	2.3	0.9	1.9	0.5	---
		5	1.9	1.6	1.7	2.3	1.6	1.7	2.3	1.6	2.3	1.2	0.9	2.6	0.6	1.2	0.4	1.9	0.6	1.8	1.1	2.0	1.2	1.8	0.9	2.1
		6	0.9●	1.2●	0.7●	1.4●	1.7●	1.9●	1.9●	1.4●	1.2●	1.7●	1.2●	1.6●	0.5●	1.3●	0.4●	1.5●	0.6●	1.6●	0.8●	1.9●	0.4●	1.5●	0.5●	1.6●
		7	2.1●		2.0●		3.1●		3.6●		4.0●		3.6●		1.1●		1.3●		1.4●		1.4●		1.3●		1.1●	
1	1538	1	1.0	1.7	1.1	0.6	1.0	1.3	1.2	0.9	1.0	1.2	2.1	1.5	0.6	0.6	1.0	0.3	0.3	1.0	1.0	1.0	1.0	0.6	1.0	1.0
		2	1.1	1.8	0.6	1.6	0.7	0.7	0.7	1.6	0.9	1.3	0.7	1.4	1.2	0.7	1.7	0.8	1.7	1.8	2.3	1.6	2.5	2.3	2.8	1.3
		3	1.3	1.8	0.9	1.1	0.9	1.7	1.7	2.6	1.7	4.0	1.7	4.1	2.0	1.9	3.1	2.5	3.2	2.8	5.6	3.0	5.2	3.0	6.2	3.4
		4	1.8	1.4	0.9	1.9	0.9	0.7	1.4	1.5	1.5	1.4	1.3	---	1.6	1.0	2.3	1.0	3.8	1.7	4.0	1.3	3.7	1.5	5.5	1.4
		5	0.9	1.2	0.7	1.6	0.8	1.6	1.8	1.5	1.7	1.5	1.6	2.4	1.3	0.7	1.7	0.6	1.8	1.2	2.7	1.7	3.3	2.0	3.1	1.8
		6	0.9●	1.2●	0.5●	1.7●	1.1●	1.4●	1.0●	0.9●	0.7●	1.2●	0.9●	1.4●	1.1●	0.7●	0.9●	0.7●	1.1●	0.8●	1.3●	1.3●	1.6●	0.9●	2.1●	1.0●
		7	1.5●		1.5●		1.7●		1.5●		1.6●		1.5●		1.0●		1.1●		1.4●		1.7●		1.4●		2.7●	
1	98	1	1.1	1.0	1.6	0.9	1.1	0.9	1.1	1.7	0.8	1.5	1.0	1.3	1.0	0.7	0.9	0.7	1.0	1.0	1.6	1.0	1.4	1.0	1.5	1.1
		2	1.1	1.4	1.3	1.2	1.1	1.4	0.9	1.5	1.0	1.9	1.6	1.4	1.0	0.8	2.5	1.7	2.6	1.9	4.2	2.4	3.2	2.2	3.3	2.2
		3	1.3	1.2	1.3	1.8	1.3	1.4	2.6	2.4	2.7	3.7	1.5	4.0	1.2	1.7	1.7	2.4	2.6	3.1	3.4	4.5	2.3	4.7	2.7	4.8
		4	1.5	1.3	0.8	1.0	0.9	1.1	1.1	1.9	1.3	1.4	0.8	---	1.3	1.4	1.4	1.9	1.8	1.9	2.3	1.6	2.2	2.0	2.1	1.7
		5	0.9	1.4	1.4	1.3	1.3	1.5	1.8	1.4	1.3	1.5	0.9	1.5	0.9	1.0	1.1	1.8	1.1	1.8	2.3	1.9	2.6	1.9	2.7	1.7
		6	0.9●	1.4●	1.0●	1.1●	1.1●	1.4●	1.2●	1.0●	1.1●	1.0●	1.0●	1.2●	0.9●	0.9●	1.2●	0.9●	0.8●	1.0●	0.8●	1.2●	1.3●	1.1●	1.3●	1.4●
		7	1.5●		1.6●		1.4●		1.3●		1.3●		1.3●		1.1●		1.2●		0.9●		1.1●		1.4●		1.5●	
1	100	1	1.1	0.9	1.0	1.0	1.0	1.0	1.2	0.9	1.3	1.1	1.0	1.0	1.1	0.7	1.3	0.9	1.1	0.8	0.8	0.9	1.3	1.1	1.2	1.1
		2	1.0	1.1	0.9	0.9	0.8	1.0	0.7	0.9	0.7	1.1	0.9	1.1	1.0	1.0	1.5	1.2	1.8	1.3	2.0	1.7	2.2	1.7	2.1	1.6
		3	1.0	1.1	0.9	1.2	1.2	1.3	1.3	2.0	1.5	1.7	1.5	1.5	1.4	1.5	2.0	1.5	3.3	2.9	3.4	3.3	3.7	3.5	3.7	2.5
		4	0.9	1.3	0.9	1.0	1.0	1.3	0.9	1.3	1.2	1.2	1.1	---	0.7	1.0	1.2	1.4	1.5	1.6	1.8	1.7	1.8	1.7	1.7	1.3
		5	0.9	1.2	0.7	1.1	0.9	1.1	1.0	1.2	1.0	1.3	0.9	1.1	0.9	1.0	1.0	1.4	1.7	1.5	2.4	2.0	3.0*	2.0	2.9	1.4
		6	0.9●	1.3●	1.0●	1.1●	0.9●	1.1●	0.9●	1.2●	0.8●	1.2●	0.9●	0.9●	1.0●	1.0●	1.0●	1.1●	1.1●	1.0●	1.1●	1.0●	1.1●	1.2●	1.6●	1.4●
		7	0.9●		1.1●		1.3●		1.3●		1.2●		1.1●		1.3●		1.2●		1.2●		1.4●		1.7●		1.9●	

● = 1, 5, 10, 50, 100, 500 μg/plt

* = 2.969

Table 16

SR1 AMES TEST DATA ON RESIDUAL OIL FRACTIONS

The numerical data is presented as R ratio values (test over control revertants)

Each fraction, except #7, was tested at two separate times, hence two values for each concentration.

OIL RO NUMBER	TESTER STRAIN	FRACTION	NO ACTIVATION												MICROSOME ACTIVATION											
			Quantity of Sample in micrograms per plate												Quantity of Sample in micrograms per plate											
			10		50		100		500		1000		2500		10		50		100		500		1000		2500	
5	1537	1	0.6	0.8	0.5	0.8	0.6	1.1	0.6	1.1	1.2	1.2	0.4	1.1	0.8	0.8	1.2	0.6	1.2	0.9	1.3	1.3	1.6	1.0	1.0	1.1
		2	0.7	1.0	1.0	0.8	0.4	0.9	1.0	1.1	0.2	1.2	0.6	1.8	1.4	0.7	2.1	1.4	2.4	1.9	4.4	1.4	2.8	1.3	3.6	1.3
		3	0.9	0.7	0.7	1.0	0.9	0.5	0.9	1.0	1.5	0.9	1.6	1.1	0.6	0.7	0.6	1.0	0.7	0.8	1.0	1.1	0.9	1.2	0.9	1.1
		4	0.9	0.7●	1.1	1.0 ●	1.1	1.2●	1.9	1.0●	2.7	1.1●	2.7	1.2●	0.3	1.3●	0.6	0.8●	0.4	1.3●	0.8	1.1●	0.7	1.1●	0.8	0.9●
		5	1.2	1.0+	2.0	1.1 +	1.2	0.7+	2.3	0.7+	2.7	0.4+	2.8	0.5+	0.6	0.6+	0.6	1.1+	1.1	0.6+	0.7	0.9+	1.0	1.1+	1.2	0.8+
		6	1.6	0.9	2.3	0.7	1.6	1.0	1.9	0.6	1.6	1.1	1.7	0.7	0.9	0.7	1.2	0.8	0.7	0.6	1.0	0.8	0.9	0.9	0.9	0.9
		7	1.6*		0.9*		0.9*		1.5*		1.5*		1.2*		0.9*		0.7*		0.7*		1.0*		1.0*		1.4*	
5	1538	1	0.4	1.4	1.0	0.4	0.6	1.3	1.3	2.1	1.0	0.9	1.3	1.8	0.6	1.0	1.0	1.2	0.6	1.5	0.6	1.3	1.0	1.3	0.7	1.8
		2	1.0	0.6	0.5	1.1	1.0	0.9	0.4	0.9	0.7	0.6	1.2	0.8	0.8	1.5	1.1	2.0	1.4	2.0	2.9	3.0	2.9	3.1	2.0	2.9
		3	0.8	0.9	1.3	1.3	1.1	1.1	2.3	2.4	3.3	2.6	2.7	1.7	1.4	1.2	2.2	1.7	2.7	2.2	4.1	3.3	3.3	2.8	4.9	2.4
		4	0.9	1.3	0.5	1.4	0.9	1.4	1.6	1.9	1.8	2.2	2.1	3.0	1.1	1.3	1.1	1.4	1.7	2.4	3.8	2.6	4.0	2.6	4.6	2.9
		5	0.9	1.0+	1.7	0.9	1.1	0.8	1.3	0.9	1.9	0.9	2.1	1.1	1.0	0.5+	1.8	1.4	3.3	1.4	4.7	1.7	4.2	2.3	4.1	3.0
		6	0.9	1.4	0.6	0.7	1.1	0.7	1.7	1.0	1.5	0.5	1.3	1.2	1.1	1.0	2.0	0.8	2.7	1.1	4.6	1.4	4.6	1.6	5.3	2.0
		7	0.9 *		0.7*		0.6*		1.1*		1.3*		1.5*		0.7*		1.0*		1.6*		1.7*		2.3*		4.8*	
5	98	1	1.0	0.7	1.1	0.6	1.0	1.0	1.1	1.0	1.0	1.0	0.8	1.0	1.3	1.1	1.0	1.1	1.3	1.1	1.3	1.0	1.1	1.1	1.0	1.0
		2	1.0	0.7	0.7	0.7	0.7	0.6	0.8	0.9	1.6	0.6	3.2	1.1	0.9	1.4	2.1	1.2	2.2	1.6	3.2	1.7	4.6	2.0	3.8	1.8
		3	0.9	1.1	1.0	0.8	1.3	1.6	4.7	3.1	8.5	4.4	5.2	4.4	1.4	1.5	2.2	2.0	2.4	2.4	2.2	2.7	1.8	3.1	3.0	2.5
		4	1.0	1.2	1.0	1.2	1.0	1.9	1.4	2.3	2.1	3.3	3.1	3.2	0.8	0.9	0.7	1.3	1.2	2.1	1.8	2.6	1.9	2.7	1.9	3.0
		5	0.8	1.0 +	1.3	1.4	1.4	1.2	1.9	1.2	1.9	1.4	1.2	1.7	1.0	1.0+	1.4	1.2	2.1	1.7	2.9	1.9	2.8	2.0	2.8	1.7
		6	1.4	1.0	1.0	1.2	0.9	1.3	1.6	1.3	1.5	1.4	1.1	1.5	1.1	0.9	1.1	1.1	0.5	1.4	1.0	1.8	1.1	2.0	1.0	2.1
		7	0.9*		0.9*		1.1*		1.0*		1.4*		1.1*		1.2*		0.9*		1.3*		1.4*		1.5*		1.5*	
5	100	1	1.0	0.7	0.9	0.7	1.0	0.6	0.7	0.6	0.8	0.6	0.9	0.8	1.0	0.6	0.9	0.7	0.8	0.6	1.0	0.7	1.0	0.6	0.8	0.6
		2	0.8	0.5	0.9	0.5	0.9	0.5	1.0	0.6	0.9	0.5	1.3	0.6	1.1	0.6	1.3	0.7	1.6	0.8	2.6	1.0	2.7	0.9	2.9	0.7
		3	0.7	0.7	1.1	0.9	1.1	0.8	1.5	1.0	1.4	1.1	1.3	0.9	1.5	1.0	2.5	1.5	3.1	1.6	3.8	1.8	3.4	1.6	3.0	1.4
		4	0.7	1.0	0.7	0.8	0.8	0.9	1.0	0.7	1.2	1.0	1.5	0.9	1.0	0.9	1.1	1.3	1.1	1.6	1.7	1.7	1.3	1.8	1.5	2.0
		5	1.0	0.8+	0.8	1.0	1.0	0.8	1.3	0.9	1.1	0.9	1.3	0.8	1.2	1.0	1.3	1.0	1.9	1.0	2.6	1.4	2.5	1.7	2.5	1.3
		6	1.1	0.9	0.9	1.0	1.2	1.0	1.3	1.1	1.2	1.1	1.4	1.0	1.1	0.9+	1.2	1.1	1.2	1.2	1.7	1.4	1.7	1.2	1.8	1.3
		7	1.0*		0.9*		1.0*		0.9*		1.2*		1.2*		1.3*		1.3*		1.1*		1.4*		1.9*		2.3*	

● = 50, 100, 500, 1000, 1500, 2500 µg/plt all strains

+ = 10, 50, 100, 250, 500, 750 µg/plt all strains

* = 1, 5, 10, 50, 100, 500 µg/plt all strains

Table 17

SR1 AMES TEST DATA ON RESIDUAL OIL FRACTIONS

The numerical data is presented as R ratio values (test over control revertants)

Each fraction, except #7, was tested at two separate times, hence two values for each concentration.

NO ACTIVATION

OIL RO NUMBER	TESTER STRAIN	FRACTION	Quantity of Sample in micrograms per plate											
			10		50		100		500		1000		2500	
12	1537	1	0.4	0.9	0.6	0.9	0.6	0.5	0.6	0.9	0.6	1.1	1.0	1.5
		2	1.0	0.8	0.5	0.9	1.2	0.3	0.2	1.5	1.0	1.2	1.3	---
		3	1.7	2.1	2.0	1.5	2.1	2.1	3.6	1.7	2.0	2.1	2.1	1.2
		4	1.6	0.6	2.5	1.6	1.6	1.1	3.3	0.9	2.9	1.2	2.1	1.8
		5	2.5	0.9	1.9	0.5	1.9	0.9	2.3	1.2	2.0	0.6	1.5	0.6
		6	2.0	1.0	1.5	0.8	1.9	1.2	2.5	0.8	2.3	0.7	3.6	0.9
		7	1.1		0.7		2.3		4.3		2.0		3.9	
12	1538	1	0.7	1.4	0.4	1.3	0.6	1.1	0.9	1.4	1.1	1.4	1.9	1.0
		2	1.0	1.2	2.0	0.7	1.3	0.6	1.3	1.4	0.7	1.4	1.0	1.0
		3	0.5	0.9	1.6	0.9	1.0	1.2	1.0	0.8	1.1	1.4	1.5	1.3
		4	1.6	1.5	1.3	0.8	1.5	0.9	1.6	1.1	1.9	0.7	2.4	1.1
		5	1.9	0.8	0.7	0.6	1.3	0.9	1.0	0.8	1.7	1.0	1.8	1.0
		6	1.4	1.2	1.1	1.0	1.3	1.0	0.9	0.8	1.0	1.2	1.3	1.0
		7	1.2		1.6		1.2		1.1		1.6		1.7	
12	98	1	1.1	1.2	1.2	0.8	1.1	1.0	0.8	1.3	1.3	1.1	1.4	1.5
		2	0.7	1.0	1.2	1.2	1.1	1.1	0.7	1.5	0.7	1.3	0.9	1.3
		3	1.4	1.5	1.1	1.2	1.4	1.0	1.1	1.2	1.8	1.3	1.1	1.4
		4	1.2	0.8	1.1	1.4	1.4	1.2	1.2	0.7	1.8	1.4	1.6	1.6
		5	0.8	1.3	1.0	---	1.3	1.1	1.1	1.4	1.5	1.4	1.3	1.2
		6	1.0	1.3	0.9	1.0	1.3	1.1	0.9	1.1	1.2	1.0	0.9	1.1
		7	1.0		0.9		1.0		0.9		0.8		0.9	
12	100	1	1.3	1.1	0.9	1.1	0.9	0.9	1.0	1.0	0.8	1.0	1.0	1.1
		2	1.2	1.0	0.8	1.0	0.9	1.0	0.6	1.0	0.8	1.0	0.9	1.2
		3	0.9	0.9	1.1	0.8	1.2	0.9	1.4	1.2	1.1	1.0	1.1	1.1
		4	1.0	0.7	1.2	0.7	1.0	1.0	1.2	0.9	1.1	0.8	1.3	0.9
		5	1.0	1.0	0.9	1.0	1.2	1.0	1.2	1.0	1.4	1.1	1.4	1.1
		6	0.8	1.1	1.2	0.9	1.0	1.0	1.0	1.3	1.0	1.1	1.1	1.0
		7	1.0		0.9		1.0		1.2		1.1		1.0	

MICROSOME ACTIVATION

OIL RO NUMBER	TESTER STRAIN	FRACTION	Quantity of Sample in micrograms per plate											
			10		50		100		500		1000		2500	
12	1537	1	0.6	0.9	1.4	1.5	1.5	1.1	1.4	1.5	1.1	0.9	1.6	1.4
		2	0.8	0.8	0.8	1.1	1.5	1.2	1.7	1.5	1.0	2.0	1.1	1.1
		3	0.9	0.9	1.0	0.9	0.8	0.9	0.8	1.2	0.9	1.5	0.9	1.2
		4	1.2	0.7	0.7	1.1	1.2	1.0	1.1	1.3	0.9	1.5	0.8	1.5
		5	0.7	0.7	1.3	1.0	0.6	1.0	0.9	1.1	0.9	0.9	0.8	0.9
		6	0.9	0.6	1.2	0.9	1.0	0.7	0.6	1.1	1.0	1.5	0.9	1.2
		7	1.4		1.4		0.9		1.1		1.0		1.3	
12	1538	1	0.6	0.8	0.8	1.0	0.6	0.8	0.6	1.1	1.4	1.4	1.4	1.2
		2	0.5	0.8	0.7	0.9	1.5	1.1	2.8	1.1	1.6	1.0	1.9	1.2
		3	1.4	1.4	1.5	1.3	1.6	1.5	2.4	1.6	5.0	1.8	3.3	2.4
		4	1.3	1.2	0.9	0.8	1.1	1.4	1.8	1.4	2.6	1.4	2.7	1.7
		5	1.4	0.8	1.4	1.1	1.8	1.0	2.4	1.5	3.1	1.7	3.9	1.7
		6	1.4	0.8	1.3	0.8	1.3	0.9	1.6	1.1	0.8	1.4	2.6	1.5
		7	1.5		1.4		1.4		1.9		1.5		3.2	
12	98	1	1.1	1.2	1.2	1.1	1.2	0.8	1.4	1.2	1.7	1.1	1.7	0.8
		2	0.8	0.7	1.0	0.9	2.0	1.2	2.6	1.4	1.8	1.8	2.4	1.4
		3	0.9	0.8	1.2	1.2	1.0	1.3	2.2	1.4	2.2	1.2	2.5	1.3
		4	1.2	1.1	1.1	1.2	1.2	1.2	1.6	0.9	1.6	0.4	2.0	1.6
		5	0.9	0.8	0.5	1.1	1.2	1.0	1.8	1.5	2.1	1.7	1.8	1.7
		6	1.3	0.8	1.1	0.8	1.1	0.9	1.1	1.1	1.2	1.4	1.5	1.5
		7	0.8		0.9		1.0		0.7		0.9		1.2	
12	100	1	0.7	1.0	0.8	1.0	0.9	0.8	0.9	1.4	1.0	1.1	0.9	1.1
		2	0.8	1.1	1.0	1.4	1.7	1.4	1.8	1.7	2.0	2.0	2.1	2.1
		3	1.1	1.0	1.4	1.1	1.8	1.4	2.2	2.2	2.9	2.4	3.3	2.5
		4	1.4	1.1	1.1	1.0	1.4	1.1	1.5	1.2	1.8	1.3	1.9	2.1
		5	1.3	0.9	1.2	1.0	1.5	1.0	2.1	1.4	2.4	1.7	2.3	1.7
		6	1.1	1.1	1.3	1.1	1.2	1.2	1.2	1.3	1.2	1.4	1.5	1.4
		7	1.2		1.1		1.2		1.3		1.0		1.4	

Table 18

SRI AMES TEST DATA ON RESIDUAL OIL FRACTIONS

The numerical data is presented as R ratio values (test over control revertants)

Each fraction, except #7, was tested at two separate times, hence two values for each concentration.

NO ACTIVATION

			Quantity of Sample in micrograms per plate											
OIL RO NUMBER	TESTER STRAIN	FRACTION	10		50		100		500		1000		2500	
14	1537	1	1.1	0.7	0.7	0.6	1.4	0.5	0.7	0.3	0.9	0.3	1.2	0.9
		2	1.0	1.0	1.5	0.4	1.0	1.3	1.3	1.2	0.9	1.3	1.0	1.6
		3	1.1	0.7	1.0	1.2	2.6	1.6	3.5	1.3	4.4	1.9	3.6	3.7
		4	1.2	0.7	1.2	0.7	1.0	1.0	1.4	1.2	1.0	1.3	2.0	1.3
		5	0.9		0.8		1.4		0.9		1.5		1.2	
		6	0.9	0.4	0.9	1.3	0.8	0.7	1.0	1.0	1.2	0.7	0.6	
		7	1.0		0.5		1.5		1.6		1.0		1.9	
14	1538	1	1.1	0.5	1.2	0.7	0.7	0.8	1.6	1.5	1.1	0.8	0.9	0.8
		2	0.6	0.8	0.9	1.1	0.3	1.1	0.6	1.3	0.7	1.4	0.9	1.0
		3	0.9	1.2	1.0	1.2	1.3	2.3	1.9	3.6	4.4	3.7	3.5	3.1
		4	0.9	0.8	1.1	1.0	0.4	1.8	1.2	1.9	1.1	1.9	1.9	1.4
		5	2.0		1.0		2.0		1.5		1.6		1.1	
		6	1.0	1.1	0.8	0.9	1.3	0.7	1.1	0.8	0.7	0.9	0.8	3.6
		7	1.2		1.6		1.0		1.2		1.3		0.9	
14	98	1	1.4	0.6	1.5	0.8	2.0	0.8	1.5	0.7	1.8	0.8	1.5	0.6
		2	1.1	0.6	0.8	0.6	0.8	0.8	1.0	0.9	1.4	1.2	1.7	0.7
		3	0.9	0.6	1.8	1.2	1.9	1.4	5.0	2.5	7.5	6.5	6.3	5.4
		4	1.3	1.0	1.3	1.0	1.0	0.9	1.5	1.4	2.4	1.5	2.3	1.6
		5	0.9		1.3		1.0		1.4		1.5		1.3	
		6	1.0	0.4	1.3	0.5	1.5	0.5	1.5	0.9	1.2	0.9	0.7	0.6
		7	1.5		1.5		1.9		1.7		1.5		1.3	
14	100	1	1.0	1.0	1.1	0.9	1.2	0.9	1.1	0.9	1.0	0.8	0.7	1.0
		2	0.9	0.7	0.8	0.6	0.8	0.8	0.8	0.7	0.8	0.9	0.8	0.8
		3	1.0	0.9	1.2	1.0	1.5	1.1	1.9	1.1	2.1	1.4	1.6	1.6
		4	1.2	0.9	1.2	1.0	1.2	1.3	1.3	1.3	1.2	1.2	1.4	1.1
		5	0.9		1.0		1.0		1.0		1.0		1.2	
		6	1.0	0.7	1.0	0.9	1.0	0.8	1.1	0.8	0.9	1.0	0.9	1.0
		7	0.8		1.0		0.9		0.9		1.0		0.8	

x - µl ppt with S9 in 5th fraction 2 5 10 20
R value 1.3 0.9 1.7 --

MICROSOME ACTIVATION

			Quantity of Sample in micrograms per plate											
OIL RO NUMBER	TESTER STRAIN	FRACTION	10		50		100		500		1000		2500	
14	1537	1	1.0	0.9	1.2	1.1	0.7	0.8	1.2	1.2	0.7	0.9	0.8	1.2
		2	0.8	1.0	1.0	1.0	0.9	1.4	0.9	1.1	0.9	1.7	1.3	2.2
		3	0.5	1.2	0.8	1.2	1.0	2.0	1.1	1.7	1.1	2.5	1.3	2.3
		4	0.5	1.4	0.9	2.3	1.0	2.0	0.9	1.7	0.9	2.6	0.9	1.8
		5	0.7		0.8		0.8		1.3		0.8		0.9	
		6	1.3	0.8	0.9	1.2	0.6	0.5	0.9	1.1	0.6	1.5	0.4	1.0
		7	0.7		0.9		0.9		0.9		0.8		0.9	
14	1538	1	0.6	1.5	0.7	1.1	1.0	1.5	0.9	1.1	1.0	1.1	1.0	1.5
		2	1.0	1.3	0.9	2.4	0.9	3.7	1.1	4.1	1.2	3.4	1.4	4.1
		3	0.8	1.1	1.0	1.7	1.5	2.7	1.7	3.5	1.9	3.9	2.3	4.3
		4	0.7	1.5	1.0	1.5	0.9	1.9	1.1	2.4	1.9	2.6	1.9	3.8
		5	0.5		1.0		1.0		1.1		1.5		1.8	
		6	0.4	0.7	0.9	0.8	0.7	0.6	1.0	1.4	1.3	1.9	1.2	2.3
		7	0.7		0.8		0.8		0.7		1.0		1.4	
14	98	1	1.3	1.7	1.4	1.2	1.0	1.3	1.0	1.2	1.4	1.8	1.4	1.3
		2	1.6	1.7	1.2	1.7	1.3	4.1	2.1	3.0	3.0	2.9	2.8	2.7
		3	1.4	1.0	1.8	2.3	1.4	2.8	3.1	5.0	3.2	6.6	3.4	7.0
		4	1.5	1.2	1.0	1.6	1.2	1.9	2.1	2.2	2.6	2.9	3.5	3.4
		5	0.8	x	1.4		1.7		2.1		2.5		2.9	
		6	0.9	1.0	1.0	1.5	1.2	1.7	1.8	1.7	2.4	2.1	2.6	1.7
		7	1.3		1.4		1.3		1.7		1.2		1.8	
14	100	1	1.0	0.8	1.2	0.8	1.1	0.7	1.2	0.9	1.3	1.0	1.4	0.8
		2	1.3	1.3	1.2	1.3	1.2	2.1	1.6	1.8	1.5	1.5	1.4	1.7
		3	1.1	1.1	1.7	1.3	1.7	1.8	2.2	2.1	2.5	2.4	2.2	2.4
		4	1.3	1.1	1.0	1.0	1.3	1.8	1.6	2.1	1.9	2.2	2.0	2.4
		5	1.0	xx	1.1		1.4		2.2		1.8		1.7	
		6	1.0	0.9	1.0	0.9	1.0	1.3	1.7	1.4	1.8	1.5	1.8	1.4
		7	1.0		1.2		1.1		1.2		1.4		1.9	

xx - µl ppt with S9 in 5th fraction 2 5 10 20
R value 1.8 1.6 2.0 2.5

Table 19

SR1 AMES TEST DATA ON RESIDUAL OIL FRACTIONS

The numerical data is presented as R ratio values (test over control revertants)

Each fraction, except #7, was tested at two separate times, hence two values for each concentration.

NO ACTIVATION

OIL RO NUMBER	TESTER STRAIN	FRACTION	Quantity of Sample in micrograms per plate											
			10		50		100		500		1000		2500	
15	1537	1	0.5	0.9	0.8	1.0	1.2	0.8	1.3	1.0	1.2	0.5	0.5	0.3
		2	0.8	0.5	0.9	1.0	0.9	0.7	0.9	0.8	1.2	1.5	1.2	1.2
		3	1.3	0.7	2.1	0.5	1.6	1.2	3.7	2.5	4.2	3.0	6.1	1.9
		4	0.4	0.7	1.0	0.7	0.5	0.9	0.4	0.7	0.9	1.0	1.6	0.7
		5	0.6	0.7	1.2	0.7	0.8	0.8	1.0	0.8	1.0	0.8	1.2	0.7
		6	0.5	0.6	0.8	0.9	1.3	0.7	1.2	0.7	1.0	0.9	1.0	0.7
		7	0.5		0.9		0.9		1.2		1.2		1.2	
15	1538	1	0.7	4.4	1.2	3.6	1.0	2.5	1.3	3.3	1.3	2.5	0.4	3.6
		2	1.0	3.1	0.6	1.6	0.9	2.4	0.8	1.5	1.0	3.5	1.4	2.4
		3	1.7	2.0	1.3	3.5	1.3	4.4	2.5	8.5	4.5	15.6	5.8	7.5
		4	0.7	1.5	0.8	2.0	1.0	3.3	1.0	3.3	0.5	2.7	1.5	2.5
		5	1.3	1.6	1.3	2.0	1.7	2.2	1.0	3.5	0.8	2.5	1.4	4.0
		6	1.0	1.6	1.0	3.3	1.2	1.3	1.9	2.4	1.0	2.2	1.4	2.2
		7	1.0		0.6		0.8		1.1		0.7		1.1	
15	98	1	1.3	0.8	1.2	1.1	1.1	1.0	1.2	1.0	0.8	1.0	0.7	1.1
		2	0.7	0.9	0.6	1.1	1.1	0.7	1.0	0.8	1.2	1.0	1.3	1.3
		3	1.2	0.8	1.9	0.9	2.5	1.2	4.1	2.2	8.3	5.3	10.4	2.8
		4	1.4	1.0	1.2	0.9	1.2	0.7	1.2	1.0	1.4	0.7	0.8	0.7
		5	1.0	0.7	1.2	0.8	1.1	2.0	1.4	0.8	1.2	1.0	1.0	0.9
		6	1.1	0.9	1.4	1.1	1.2	0.9	1.4	1.0	1.1	0.9	0.8	0.9
		7	0.9		0.8		1.7		1.3		1.5		0.7	
15	100	1	1.0	0.9	1.0	1.0	0.9	0.9	0.8	1.0	0.8	1.0	0.8	1.1
		2	1.0	1.0	1.0	0.9	0.7	0.9	0.9	0.9	0.8	0.7	0.7	0.9
		3	1.1	1.1	1.0	1.4	1.5	1.4	1.8	1.4	2.1	1.6	2.0	1.3
		4	0.9	1.1	0.8	1.0	0.8	1.0	0.8	1.0	1.0	1.1	1.0	1.0
		5	0.9	1.5	1.0	1.1	1.0	1.1	1.0	1.0	1.1	1.1	1.1	1.3
		6	0.9	1.4	0.9	1.2	1.0	1.2	1.0	1.1	1.0	1.1	0.9	1.3
		7	1.0		1.0		1.0		1.2		1.1		1.0	

MICROSOME ACTIVATION

OIL RO NUMBER	TESTER STRAIN	FRACTION	Quantity of Sample in micrograms per plate											
			10		50		100		500		1000		2500	
15	1537	1	0.8	0.6	0.8	1.1	0.9	1.2	0.6	0.9	0.5	1.3	0.6	1.0
		2	0.8	1.1	0.8	1.1	1.2	1.3	1.0	1.4	0.8	1.6	1.0	1.7
		3	0.6	0.6	0.7	0.9	0.8	0.9	2.0	1.2	2.5	1.3	2.8	1.0
		4	0.4	0.9	0.6	0.9	0.7	1.1	0.8	---	0.8	1.2	0.9	0.9
		5	0.5	0.7	0.6	1.0	0.6	0.9	0.7	0.8	1.1	0.7	1.0	0.8
		6	0.9	0.6	0.9	0.5	0.8	0.6	1.0	0.7	0.7	0.9	1.1	1.3
		7	1.0		0.9		0.9		1.1		0.9			
15	1538	1	0.4	0.9	0.5	0.8	0.5	1.0	0.8	0.7	0.6	0.9	1.0	1.0
		2	0.9	1.4	0.9	1.4	1.0	1.2	1.6	2.4	2.2	2.8	2.6	2.9
		3	0.7	0.5	1.2	1.1	1.5	1.2	2.4	2.0	2.5	3.1	3.0	2.7
		4	0.9	0.8	0.6	0.7	1.0	0.9	1.0	0.9	1.5	1.3	1.6	1.3
		5	0.5	0.7	0.8	0.8	1.0	1.0	1.4	1.3	2.0	1.9	2.0	1.4
		6	0.9	0.6	0.5	1.0	0.5	0.9	0.4	0.9	1.2	0.8	1.5	1.1
		7	0.8		0.8		0.6		0.8		0.8		0.7	
15	98	1	1.2	1.0	0.9	0.7	1.0	1.2	1.3	1.2	1.5	1.0	1.4	1.3
		2	1.0	1.5	1.4	1.6	1.3	1.6	2.3	2.2	3.1	2.6	3.7	2.5
		3	1.6	1.3	2.6	1.0	3.0	2.2	5.0	3.1	6.5	3.1	8.5	2.7
		4	1.2	1.0	0.9	0.7	1.0	1.2	1.4	1.4	2.6	1.3	2.2	1.7
		5	1.2	1.1	1.6	1.0	1.7	1.2	2.3	1.3	2.9	1.6	2.5	1.6
		6	1.2	0.7	0.9	1.0	1.0	1.0	1.5	1.2	2.3	1.6	2.0	1.6
		7	1.2		1.0		1.1		1.3		1.4		---	
15	100	1	1.2	1.0	1.2	0.8	1.2	0.8	1.2	1.0	1.3	1.1	1.2	0.9
		2	1.0	1.2	1.2	1.0	1.4	1.1	1.9	1.6	2.0	1.6	1.6	1.7
		3	1.3	1.3	1.9	1.3	2.7	1.8	3.1	2.0	3.2	1.8	2.8	1.8
		4	1.0	0.9	0.9	1.0	1.0	1.5	1.4	1.5	1.6	1.6	21	1.5
		5	1.0	0.9	1.0	1.1	1.6	1.1	2.0	1.4	2.5	1.5	2.0	1.6
		6	1.3	1.0	1.1	1.0	1.0	1.1	1.3	1.1	1.5	1.3	1.4	1.4
		7	1.0		1.0		1.2		1.3		1.1		1.1	

Table 20

SR1 AMES TEST DATA ON RESIDUAL OIL FRACTIONS

The numerical data is presented as R ratio values (test over control revertants)

Each fraction, except #7, was tested at two separate times, hence two values for each concentration.

OIL RO NUMBER	TESTER STRAIN	FRACTION	NO ACTIVATION Quantity of Sample in micrograms per plate												MICROSOME ACTIVATION Quantity of Sample in micrograms per plate											
			10		50		100		500		1000		2500		10		50		100		500		1000		2500	
16	1537	1	0.6	0.9	1.1	0.9	0.6	0.9	0.9	1.5	0.9	1.5	1.0	0.8	0.7	1.6	0.3	1.6	0.6	1.9	0.5	1.4	0.5	1.5	0.5	1.5
		2	0.5	2.5	0.5	1.4	0.4	1.3	1.0	1.8	0.5	2.1	0.6	0.9	0.8	0.9	0.3	1.4	0.6	1.4	1.3	1.7	1.2	1.3	1.4	1.5
		3	1.1	1.6	0.7	2.7	1.2	1.7	1.6	5.3	2.9	3.4	4.5	2.6	1.0	0.9	1.0	1.4	1.5	2.5	1.0	2.8	2.0	3.8	2.2	1.8
		4	1.2	1.6	0.9	1.5	1.5	1.8	2.0	1.7	2.6	2.2	2.5	2.4	0.5	1.6	0.6	1.2	1.1	1.7	1.5	2.1	2.1	2.2	2.4	1.8
		5	0.5	1.7	1.4	1.6	1.5	1.8	1.5	2.7	2.0	2.6	1.9	---	0.7	1.0	0.9	2.1	1.0	1.5	1.2	2.1	0.9	2.1	1.4	1.5
		6	---	1.3	1.0	1.5	1.8	0.6	2.3	1.4	1.2	1.3	1.2	1.4	0.6	1.4	0.5	1.4	0.6	1.6	0.8	1.6	0.8	1.9	0.8	1.6
		7	1.2		1.4		1.2		0.9		1.8		0.9		1.0		0.8		0.7		0.9		0.8		0.7	
16	1539	1	1.3	1.0	0.9	1.1	0.6	C.9	1.1	1.2	1.4	0.9	1.1	1.2	1.0	1.6	0.6	1.2	0.3	1.2	0.3	1.2	1.0	1.2	1.0	1.1
		2	0.5	1.7	0.6	2.6	9.8	2.9	1.2	3.4		4.1		3.5	1.4	1.2	1.4	1.5	1.4	1.7	2.3	2.0	1.4	2.1	1.4	2.2
		3	1.1	1.1	1.8	1.4	1.2	1.1	2.4	1.8	4.8	2.7	4.3	2.4	1.1	1.2	1.7	1.7	1.8	3.0	1.7	3.5	2.7	3.8	3.2	3.9
		4	1.0	0.8	1.0	1.5	1.0	1.3	1.7	1.2	1.2	1.5	2.2	2.3	0.9	1.6	0.7	1.6	0.8	1.2	1.3	2.0	2.0	1.8	3.7	2.8
		5	1.0	1.1	1.5	1.6	1.2	1.1	1.0	1.3	1.6	2.4	1.0	---	0.9	0.8	1.0	1.3	1.6	1.3	1.4	2.7	2.0	2.7	2.0	2.7
		6	1.0	0.9	1.3	0.9	0.9	1.1	1.5	0.9	1.5	1.5	0.7	1.5	0.8	1.6	0.9	1.0	0.9	1.6	1.4	2.2	1.8	2.5	2.0	3.5
		7	1.3		1.0		1.0		1.6		1.0		1.1		0.8		0.8		0.5		0.8		0.8		1.2	
16	98	1	0.5	1.2	0.9	1.7	0.6	1.0	0.6	1.2	1.1	1.4	0.7	1.3	1.5	1.3	1.3	1.1	1.0	1.1	1.0	1.3	1.3	1.1	1.0	1.1
		2	0.6	1.4	0.3	1.0	0.4	0.8	0.5	1.0	0.3	1.6	0.9	1.5	1.0	1.0	1.3	1.6	1.5	1.7	2.3	2.3	2.6	2.2	1.7	1.7
		3	1.3	1.3	1.6	1.5	1.9	2.0	4.3	2.9	7.8	3.1	8.3	3.3	1.3	1.4	2.5	2.1	3.3	3.6	3.3	4.1	4.2	4.1	5.4	4.1
		4	0.8	1.1	1.1	1.4	1.4	0.9	2.0	1.5	2.4	2.4	4.1	2.9	1.0	1.3	1.5	1.1	1.8	1.3	3.1	2.1	3.5	2.5	4.5	2.0
		5	1.5	1.7	1.2	1.4	1.2	1.4	2.0	1.6	2.5	1.6	2.7	1.7	1.3	1.0	1.9	1.3	2.1	1.5	2.6	2.1	2.3	2.1	2.4	1.9
		6	1.2	1.4	1.0	0.9	1.5	1.4	1.5	1.2	1.2	1.1	1.0	1.6	1.1	1.1	1.3	1.3	1.5	1.4	2.3	1.6	3.2	1.9	3.8	2.3
		7	0.8		1.3		1.0		1.0		---		0.8		1.2		0.9		1.3		1.2		2.3		1.7	
16	100	1	0.9	0.9	0.9	1.0	0.9	0.9	0.8	1.1	0.9	0.9	0.9	1.0	1.0	1.1	1.0	0.9	0.9	1.1	1.0	1.1	1.1	1.2	1.0	1.3
		2	0.8	0.9	0.7	0.9	0.7	0.7	0.7	0.8	0.6	0.7	0.6	0.7	0.8	1.1	0.9	1.3	1.0	1.5	1.0	2.3	1.2	2.3	0.7	2.2
		3	1.0	1.0	1.1	0.9	1.3	1.0	1.6	1.1	1.7	1.2	1.4	1.1	1.5	1.4	2.6	2.5	3.2	3.3	3.1	4.2	3.2	4.2	4.5	4.6
		4	0.9	1.0	1.1	1.2	1.0	1.1	1.4	1.3	1.7	1.4	2.0	1.7	1.2	1.3	1.4	1.5	1.4	1.3	2.1	2.7	2.7	3.4	3.2	2.7
		5	1.0	1.0	0.9	1.2	1.1	1.2	1.2	1.3	1.5	1.4	1.2	1.5	1.0	1.3	1.5	1.6	1.7	2.3	2.0	2.4	2.2	2.3	2.0	1.6
		6	1.0	1.0	1.0	0.9	1.3	1.2	1.2	1.2	1.2	1.3	1.0	0.9	1.2	1.2^{x}	1.3	1.5	1.4	1.5	2.4	2.3	2.9	2.3	2.8	1.9
		7	1.2		1.1		1.2		1.2		1.0		1.2		1.2	xx	1.3		1.3		1.2		1.3		1.6	

^{x} µl of ppt 10 20 30 50
R value .9 1.3 1.7 1.8

^{xx} µl ppt 10 20 30 40 50
R value 1.0 .8 1.0 1.3 1.5

Table 21

SRI AMES TEST DATA ON SEVEN FRACTIONS OF SIX OIL SAMPLES

Only positive response is recorded. All fractions had two test runs except #7, which had only one run due to lack of sample. Question mark (Q) means a positive, not dose related, response recorded. P means the average of two tests is positive.

No Activation

		TA1535	TA1537		Avg	TA1538		Avg	TA98		Avg	TA100		Avg
1	1													
	2													
	3			Q			+		+		P			
	4													
	5													
	6													
	7		+		P									
5	1													
	2								+					
	3					Q			+	+	P			
	4						Q		+	+	P			
	5													
	6													
	7													
12	1													
	2													
	3		Q											
	4		Q											
	5													
	6		+		P									
	7		+											
14	1													
	2													
	3		+	+	P	+	+	P	+	+	P			
	4													
	5													
	6					+								
	7													
15	1					+								
	2					Q								
	3		+	+	P	+	+	P	+	+	P			
	4					+								
	5					+								
	6					Q								
	7													
16	1													
	2													
	3		+	+	P	+		P	+	+	P			
	4						+	P		+	P			
	5													
	6													
	7													

Microsome Activation - S9

		TA1535	TA1537		Avg	TA1538		Avg	TA98		Avg	TA100		Avg
1	1													
	2								+		P			
	3			+		+	+	P		+	P	+	+	P
	4					+								
	5					+						Q		
	6													
	7													
5	1													
	2		+			+	+	P	+		P			
	3					+	Q	P		+		+		
	4					+		P		+				
	5					+	+	P						
	6					+		P						
	7					+		P						
12	1													
	2													
	3					+		P				+		
	4													
	5					+								
	6													
	7					+		P						
14	1													
	2					+			+	+				
	3					+		P	+	+	P			
	4					+			+	+	P			
	5													
	6													
	7													
15	1													
	2									+	P			
	3		Q	+					+	+	P	+		
	4													
	5													
	6													
	7													
16	1													
	2													
	3		Q			+	+	P	+	+	P	+	+	P
	4						+	P		+	P	+	+	P
	5													
	6					+				+	P			
	7													

Table 22

SUMMARY OF SRI AMES TEST DATA ON OIL FRACTIONS

Results presented as R value averages of 2 tests, except fraction #7, single test.

	NO ACTIVATION					ACTIVATION-S9			
RO #	TA1537	TA1538	TA98	TA100		TA1537	TA1538	TA98	TA100
1					FRACTION #1				
5									
12									
14									
15									
16									
1					FRACTION #2			+	
5							+	+	
12									
14									
15								+	
16									
1			+		FRACTION #3		+	+	+
5			+				+		
12							+		
14	+	+	+				+	+	
15	+	+	+					+	
16	+	+	+				+	+	+
1					FRACTION #4		+		
5			+				+		
12									
14								+	
15									
16		+	+				+	+	+
1					FRACTION #5				
5							+		
12									
14									
15									
16									
1					FRACTION #6				
5							+		
12									
14									
15									
16								+	
1	+				FRACTION #7				
5									
12	+								
14									
15									
16									

agreement that fraction #1 is not mutagenic in any of the six oils. From then on each oil will be discussed separately. It is again shown that all tests with TA 1535 are negative and hence this strain will not be further mentioned.

RO #1

Fraction 2. SRI concludes mutagenic response with activation due to dose related increase in TA 1538, 98, and 100. Our evaluation agrees with no mutagenicity without metabolic activation. With S9 MIX we consider TA 100 negative. TA 1538 is considered negative, although the first run has a dose response increase to almost double that of the control, but the second has no increase. TA 98 is positive in the first run, negative the second. We judge RO 1-2 to be not mutagenic.

Fraction 3. SRI states TA 1537, 1538, 98, and 100 show mutagenicity. In the first test only TA 98 and 100 are mutagenic without activation, in the second test all four strains are positive. With activation, the first test is positive for TA 1538, 98, and 100 and in all four strains in the second run. In our evaluation all strains are negative without activation in the first run, positive in the second, except for TA 100. With activation first run is positive for TA 1538, 98, and 100, run number two is mutagenic in all four strains.

Fraction 4. SRI concludes that TA 98 and 100 are mutagenic with metabolic activation. We judge TA 98 and TA 100 as negative but TA 1538 positive in the first test only, giving an inconclusive response.

Fraction 5. SRI calls TA 1538, 98 and 100 positive with S9 MIX. Our evaluation shows TA 1538 and 100 positive only in the first test, TA 98 positive in the first test and dose response increase only in the second test. This fraction is evaluated as not mutagenic by us.

Fraction 6. SRI calls it mutagenic with activation in TA 100 and possibly TA 1538 and 98. We find no dose related increase to indicate even weak mutagenicity.

Fraction 7. SRI has mutagenicity with activation in TA 1538, 98, and 100, opposed to our negative evaluation with a very weak TA 1538 dose response. However, we find TA 1537 positive without activation.

Thus in summary our verdict is that without activation Fraction 7 is mutagenic in TA 1537, and Fraction 3 with a negative-positive reaction in TA 1537, 1538, and 98. With S9 MIX fraction 3 is positive in TA 1538, 98, and 100 in both tests. Fraction 2 in TA 98, Fraction 4 and 5 in TA 1538 are positive-negative, in first and second test respectively, also Fraction 5 in TA 100, while TA 1537 is positive in Fraction 3 in the second test.

RO #5

Fraction 2. All four strains are considered mutagenic by SRI with activation. We judge TA 1537, 38, and 98 positive in the first test and TA 100 questionable, while in the second test only TA 1538 gives a mutagenic response. We also note that without activation TA 98 is positive in the first run.

Fraction 3. Without activation TA 1538 and 98 are called mutagenic by SRI with TA 100 showing elevated counts. With S9 MIX TA 1538, 98, and 100 are positive. Our evaluation of the results calls only TA 98 as positive without activation, while with activation TA 1538 and 98 are weakly positive and TA 100 gives a positive-negative response.

Fraction 4. Here SRI evaluates the same response as for Fraction 3. Our analysis is also the same as above except that with activation TA 100 is negative. There is however a dose response increase without activation in TA 1537 in the first run.

Fraction 5. Here also TA 1538, 98, and 100 are judged by SRI mutagenic with S9 MIX and some revertant elevation in TA 1538, 98 without activation in the first test. Our analysis shows only TA-1538 as positive with activation, all other strains as not mutagenic. There is a dose response increase without activation in TA 1537 in the first run.

Fraction 6. Conclusion is that with activation TA 1538 and 100 has mutagenic activity and TA 98 a possibility. We find only TA 1538 with a positive response on the first test, negative in the second.

Fraction 7. This fraction, due to lack of sample, was tested only once. SRI calls it positive with S9 MIX in TA 1538, 98, and 100, while our evaluation shows only TA 1538 to be such.

RO #12

Fraction 2. SRI finds with activation a dose related increase in TA 98 and 100 and elevated counts in TA 1538 in the first test and TA 1537 for the second test. We find all results negative. With activation there is some dose response increase in the first run in TA 1538 and 98.

Fraction 3. The subcontractor's analysis is that no activation produces an irregular increase in TA 1537, 1538, and 98 and that with activation a mutagenic activity is present in TA 1538, 98, and 100. We find that there is a possible positive response in TA 1537 first test without activation. With S9 MIX TA 1538 and 100 are positive-negative, TA 98 has only a dose related increase in the first test.

Fraction 4. With activation TA 1538, 98, and 100 have mutagenic activity while without activation there are increases in TA 1537, and in TA 1538 and in TA 98 there is irregular elevation. Our analysis shows that without activation TA 1537 is positive in the first test only. All other tests are negative with some dose related elevation in TA 1538 with activation.

Fraction 5. SRI has this fraction with mutagenic activity in TA 1538, 98, and 100 with S9 MIX and some elevation without the enzyme system. We find only TA 1538 positive in the first test with activation. All other values are negative and without discernable elevations.

Fraction 6. SRI concludes that a weakly mutagenic action is present with activation in TA 1538 and TA 100 and possibly in TA 98. We find no such activity, but TA 1537 is positive in the first test without the S9 MIX.

Fraction 7. Elevated number of revertants with S9 MIX in TA 1538. 98, and 100 are reported. We find that TA 1537 without activation has a mutagenic activity as does TA 1538 with activation. All other tester strains show a negative response.

RO #14

Fraction 2. Mutagenic activity with activation is reported in TA 1538, 98 and 100 and with elevated counts in TA 1537. With S9 MIX we find that TA 1538 is positive in the second test only, TA 98 shows mutagenic activity, while TA 100 is negative.

Fraction 3. SRI judges this fraction with and without activation positive in all four tester strains. Without activation TA 100 should be classed negative. With activation TA 1537 and TA 100 are both negative.

Fraction 4. With activation a dose related reproducible increase in revertants is demonstrated in TA 1538, 98, 100. We find only TA 98 positive with TA 1538 showing mutagenic activity in the second test.

Fraction 5. SRI analyses positive response with activation in TA 1538, 98, and 100. We find all samples negative, with dose related increase in TA 98 in the first run with S9 MIX.

Fraction 6. Conclusion is that with activation TA 98 and 100 are mutagenic. We find all activated strains negative. Without activation TA 1537 is positive in the second test.

Fraction 7. A dose related increase with activation is reported in TA 100 and elevation of revertant counts in TA 1538 and 98. Our analysis evaluates all responses as negative.

RO #15

Fraction 1. Although this fraction is negative in all other situations and is so classified by SRI, a positive response is shown in TA 1538, second test, no activation.

Fraction 2. Conclusion is that this fraction is a frameshift mutagen with activation in TA 1538, 98, and 100 and has a dose related increase in TA 1537, second test. Our analysis shows TA 1538 to be positive without activation in second test. With activation only TA 98, first test is positive, although a dose related increase is seen in the second test and in both tests with TA 1538.

Fraction 3. This fraction is mutagenic in all four strains with and without activation. We do not find TA 100 without activation to be mutagenic. With S9 MIX TA 1537 has a dose response increase in the first test and TA 100 has a positive result. Both strains are negative on the second test.

Fraction 4. SRI judges TA 1538, 98, and 100 to have mutagenic activity with activation. We find only TA 1538 in the second test without activation to be positive. There is a dose related increase in TA 98 in the first test with activation.

Fraction 5. The same response as in Fraction 4.

Fraction 6. With activation, dose response in TA 98 and 100 and activity in first test at high doses in TA 1538. We find no activity with S9 MIX portion, however, TA 1538 without S9 MIX could be showing mutagenic activity in the second test.

Fraction 7. SRI observes a small revertant increase with activation of TA 98 and 100. It is not, however, mutagenic. We do not observe any increase.

RO #16

Fraction 2. Conclusion is that mutagenic activity with activation is present in TA 1538, 98, and 100. We find no such activity. Only in TA 1538 without activation, the second test is positive.

Fraction 3. This fraction is called by SRI as positive in with and without metabolic activation assays. Our analysis of the assay data shows that without activation only TA 1537 and 98 are clearly positive, while TA 1538 as such is positive in the first test only, and TA 100 not at all. With activation TA 1538, 98, and 100 are positive, but TA 1537 shows only a questionable positive response in the second test.

Fraction 4. With activation mutagenic activity present in all four tester strains, without activation TA 1538 is negative. Our evaluation finds that without S9 MIX only activity is in TA 98, where the first test is positive, the second has a dose response curve. With activation TA 100 is positive, TA 98 only so in the first test. TA 1537 and 1538 are both negative.

Fraction 5. Both tests with activation have a dose related increase in TA 1538, 98 and 100 and in the second test also for TA 1537. We find all strains without mutagenic activity.

Fraction 6. SRI evaluation is the same as for Fraction 5. Our analysis is also the same except that with S9 MIX, in the first test TA 98 was positive.

Fraction 7. Mutagenic with activation in TA 100 and possibly TA 98. We find no increase in counts, no mutagenic activity.

Conclusions on Oil Fraction Analysis with Discussion

- Tester strain TA 1535 gives negative response, therefore, should not be considered for future tests.
- Fraction #1 need not be tested, because the response is negative.

- Fraction #7 cannot be judged negative, however, the positive response in RO #1 and 12 without activation is based on a single test and the R values are questionable. A retest may be considered, but since the three preceding fractions, #4, #5 and #6 are TA 1537 negative without activation, it may be that these two positive responses are not true ones.
- Fraction #3 contains mutagenic compounds with and without activation.
- Without activation in two oil samples, RO #5 and RO #15, the fraction #3 activity is carried over to fraction #4.
- With metabolic activation positive response starts in fraction #2, is at maximum in fraction #3 with carry-over to fraction #4. RO #5 carried mutagenicity through fractions #5 and #6.
- Tester strain TA 100 is positive on three occasions with activation. In all three cases TA 1538 and TA 98 are also positive. TA 100 is negative without activation, hence this strain can be omitted in future tests.
- Strain TA 1537 is negative with activation and positive, except for fraction #7 discussed above, only for fraction #3 when not activated. In these instances TA 1538 and TA 98 are also positive, hence the use of TA 1537 for testing appears to be superfluous.
- For Ames testing of oil sample fractions only tester strains TA 1538 and TA 98 appear to be necessary.

General Data Discussion

In the 12 oil sample analyses and their comparison between W and SRI results, differences are present. There are also marked, between-test differences in the SRI data. In these cases, as with W tests, only positive oil samples responded with variations. The negative samples, W and SRI or between tests at SRI show good correlation. The negative sample, RO #12, has a very good agreement for a biological test system. This same type of a between-test difference is observed in the fraction results reported by SRI. As a representative case for this brief discussion, the R value results from RO #14, fraction #2 with microsome activation in TA 1538 is presented.

Quantity of sample per plate in micrograms, with test #1 and #2 results and the average (A) of both under each quantity.

10			50			100			500			1000			2500		
1	2	A	1	2	A	1	2	A	1	2	A	1	2	A	1	2	A
1.3	1.0	1.2	2.4	.9	1.7	3.7	.9	2.0	4.1	1.1	2.6	3.4	1.2	2.3	4.1	1.4	2.

As can be seen, test #2 has constant, negative values, test #1 is positive, but not with a good dose-response. The average of both tests is negative. The first reaction is to blame improper or inconsistent testing techniques for these variations. This is not the answer because, firstly there is good correlation on non-mutagenic samples, and secondly the positive controls respond correctly, hence, the tester strains are performing correctly. The oil slick formed on plates at higher concentrations does interfere with automated (Biotran II) revertant counting. This has been recognized and hand (visual) counting is now used. Therefore, this is not a factor in data variation. The only reasonable explanation is that with time the oil sample or its specific fractions change chemically from a non-mutagen to a mutagen. The positive versus negative response of two tests performed at different times would tend to support this hypothesis. In addition, there is published information[1] that aromatic hydrocarbons will change without microsome activation in time from a non-mutagen to a frameshift mutagen in TA 1537, 1538 and 98.

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[1] Gibson, T. L., Smart, V. B. and Smith, L. L. (1978) Non-enzymatic activation of polycyclic aromatic hydrocarbons as mutagens. Mutation Res. 49, 153-161.

It is known that certain polycyclic aromatic hydrocarbons are mutagens. Ordinarily these have molecular weights in the intermediate range (out to about 252 as for benzopyrene). Such materials could be expected to be in fraction #3 (as carry-over?), though they would be expected to be more prevalent in fraction #2 of a residual oil. The inescapable conclusion would seem to be that the major mutagens in a residual oil are not polycyclic hydrocarbons.

From information in this report it appears that the health hazard aspect of residual oil is implied as to the source, but the true nature not identified. It does, however, indicate how a health hazard program could be planned to minimize or even eliminate risk from handling of residual oil samples as through diluting or neutralizing the active components as found in fraction #3.

Volatile Fractions of Residual Fuel Oils

The volatile fraction of residual oils RO #3, #11 and #24 [see below] was dissolved at 25 mg concentration per ml of p-dioxane. The Ames test bioassay history of these three samples as the original oils is as follows: RO #3 positive with activation in TA 1538 and TA 98 by Stanford Research International, Inc. (SRI), negative by Westinghouse W retest. No response without activation. RO #11 was only tested by SRI and found to be negative in response. RO #24 gave a positive response in TA 1538 and TA 98 with and without metabolic activation in SRI tests, but was positive only with activation in TA 98 in W tests.

The volatile fraction was negative in both tester strains, TA 1538 and TA 98, with and without activation. Cytotoxicity became pronounced at 1250 μg/plate test level, especially in TA 98. From these results it is concluded that the volatile residual oil fraction does not contain the mutagenic compounds, but does carry cytotoxic moieties. The results are given in Table 23.

Table 23

VOLATILE FRACTION AMES TEST RESULTS

Numbers represent revertant counts.
Control is with corresponding volume
of p-dioxane. T = toxic

			TA 1538				TA 98			
			No Activ.		Activ.		No Activ.		Activ.	
Oil No.	Plate	Plate	Control	Ave. 3 Plates	Control	Ave. 3 Plates	Control	Ave. 3 Plates	Control	Ave. 3 Plates
3	125	5	18	23	39	50	28	33	63	65
	250	10	18	24	39	43	28	29	63	63
	500	20	22	24	38	38	36	26	51	55
	1250	50	21	20	36	31	21	26	49	41
11	125	5	18	21	39	42	28	34	63	71
	250	10	18	25	39	39	28	33	63	57
	500	20	22	20	38	39	36	24	51	47
	1250	50	21	23	36	35	21	T	49	T
24	125	5	18	21	39	47	28	36	63	63
	250	10	18	22	39	42	28	36	63	64
	500	20	22	21	38	33	36	34	51	61
	1250	50	21	23	36	36	31	22	49	T

Ten Fractions of No. 3 Fraction of An Oil Sample

In a further attempt to separate the mutagenic fraction, RO #16, the most biologically active sample, was selected for further study. From the bioassay of the seven chromatographic fractions it was determined, that the fraction #3 is the most active. Therefore, this fraction was further broken down. The column chromatography of the oil was performed as usual but instead of collecting 600 ml of CH_2Cl_2 eluant *en toto* for fraction #3, 10 - 100 ml aliquots were collected. This led to what we call ten sub-fractions of the No. 3 fraction. These were bioassayed by Westinghouse. The results are presented in Appendix D. The R values are summarized in Table 24. Without activation a certain amount of toxicity was observed and lack of sample precluded using more than one concentration. With microsome activation a positive response is present in all but the last (No. 10) fraction. The S9 MIX appears to have somewhat ameliorated the toxic effects, which become evident again at 900 µg per plate levels. The 90 µg per plate level is not reactive, except TA 98, fraction #4. It is also interesting to observe that TA 98 is more responsive to these fractions in contrast to TA 1538 which gave higher and more positive readings in the previous tests. The conclusion from this work was that this subfractionation did not really serve to elucidate residence of mutagenicity of an oil.

Dimethyl Sulfoxide Partitioning

Fractions from the DMSO partitioning of W residual oil sample #16 were subjected to Ames test at Westinghouse:

Fraction	µg/plate	TA 1538	TA 98
DMSO insoluble	90	.6	1.6
	450	1.5	2.8
	900	1.5	2.4
DMSO soluble	90	2.8	4.8
	450	2.8	3.7
	900	1.9	2.7
DMSO sol/Hexane Sol.	90	1.5	3.8
	450	3.0	11.1
	900	2.4	7.8

Table 24

Ten Fractions of No. 3 Fraction of RO 16 Oil with Microsome Activation. 9 mg/ml in p-dioxane.
Numbers represent the Test to Control revertant ratio.
A ratio ≥3.0 constitutes a positive response.
nt = not tested due to lack of sample.

TA 1538

Vol. µ	Quant. µg	1	2	3	4	5	6	7	8	9	10
10	90	1.2	1.4	1.5	1.6	1.3	1.3	1.8	1.4	nt	nt
50	450	3.7	3.0	3.8	4.1	3.9	4.6	3.0	4.1	nt	nt
100	900	3.2	2.7	3.0	3.0	3.5	3.5	2.0	2.9	5.3	1.7

TA 98

		1	2	3	4	5	6	7	8	9	10
10	90	2.2	1.8	1.9	3.2	2.6	2.5	nt	3.1	nt	nt
50	450	7.2	5.4	6.8	9.2	7.0	7.9	nt	5.4	nt	nt
100	900	5.1	3.6	6.4	6.8	5.5	6.5	nt	5.9	nt	nt

From these results it would appear that the mutagenic fraction in oil RO #16 resides in the DMSO soluble portion. The TA 98 strain is more responsive to the fractions, the DMSO/hexane soluble one having the highest activity. This is in contrast to all previous bioassays, in which TA 1538 was more responsive.

Dimethyl Sulfoxide Partitioning/Column Chromatography Pre-separation

The magnitude of the effect of DMSO partitioning/column chromtography is illustrated in Figure 50 which compares data, as available, for column chromatography fractions of as received oil RO #16 of DMSO solubles of oil RO #16 and of DMSO insolubles of oil RO #16. A summary of the data based primarily on this figure would state:

1. The DMSO insolubles from residual fuel oil RO #16 are non-mutagenic and the 7 fractions from column chromatography of these insolubles are non-mutagenic.
2. The DMSO solubles from residual fuel oil RO #16 are mutagenic and all 7 fractions from column chromatography of these solubles are mutagenic with activation in at least one concentration.
3. The mutagenicity of some fractions in 2, above, are at the highest levels of any oil or fraction tested previously under this contract. [This includes fractions from as received oil RO #16.]
4. Although our fraction 3 has appeared to be the most mutagenic across all residual fuel oils tested as fractions, data obtained through DMSO partitioning suggests other fractions such as fraction #4 may be more mutagenic, with some factor such as toxicity perhaps associated with the higher dosage levels. It is only through the greater responses obtained with DMSO use that this possible pattern emerges.

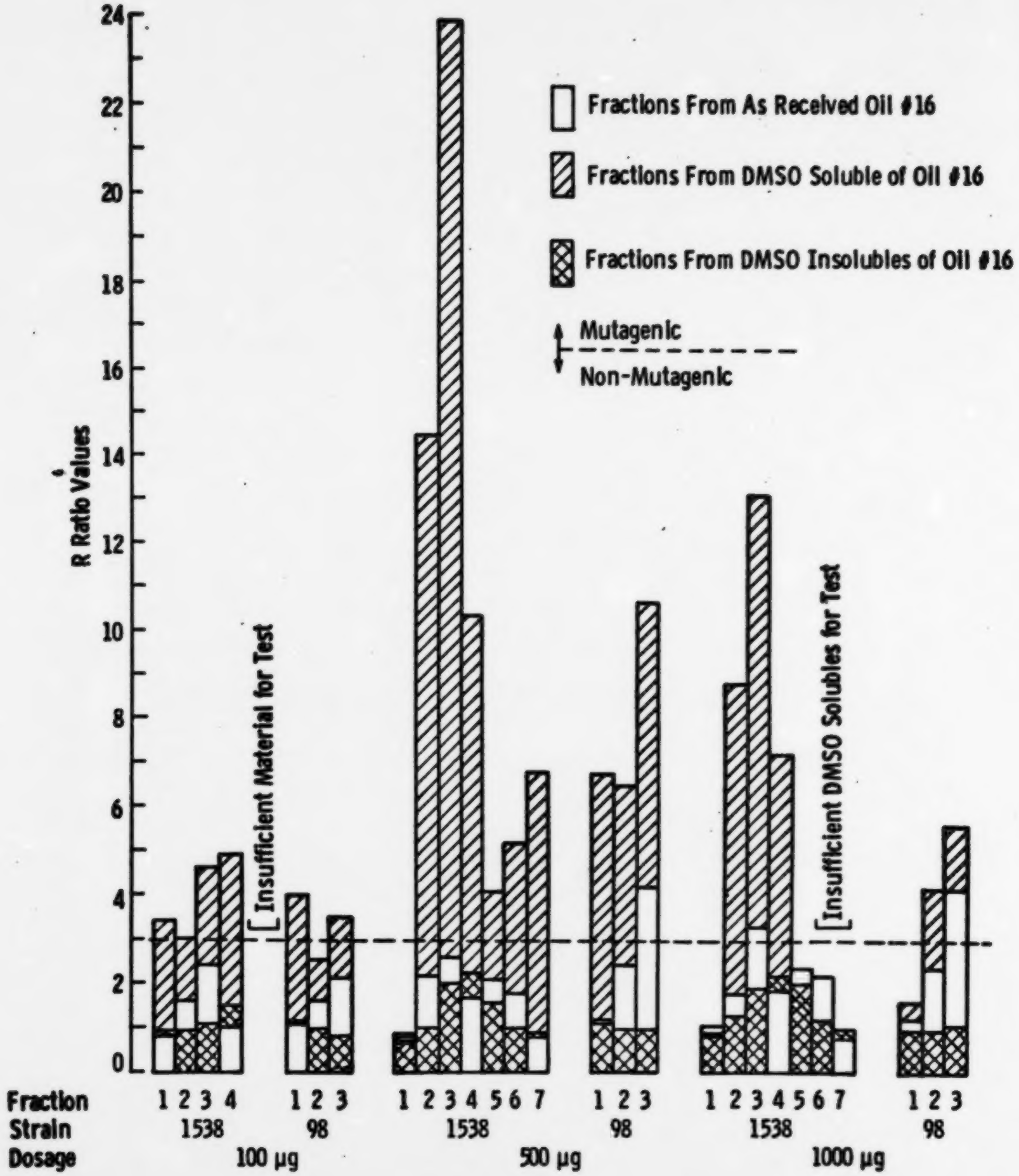


Figure 50. Comparative Ames Test Results with Microsome (S9) Activation

Figure 50 presents an additional useful treatment of bioassay data. As available, comparable data among the 7 fractions generally derived from oil RO #16 (as received, DMSO solubles and DMSO insolubles) are given. As expected, the response of the various samples follows the relationship:

$$R_{\text{DMSO Solubles}} > R_{\text{As Received}} > R_{\text{DMSO Insolubles}}$$

Variants from the above relationship involve only low responses and degrees of variance expected to be well within reproducibility limits. It is possible, however, that even within this latter caveat, fraction #4 is displaying the relationship:

$$R_{\#4\text{ from DMSO Solubles}} > R_{\#4\text{ from DMSO Insolubles}} > R_{\#4\text{ from As Received}}$$

If true this could indicate that some components of oil RO #16 are undergoing some effect such as reacting with DMSO to produce DMSO-insoluble material that may be slightly more responsive than fraction #4 from as received oil. The resultant would still be below the "mutagenicity" level.

The above findings are based on only one sample and any future work must involve additional samples to be tested to verify the results of this study. Even so, the findings appear to be rather important. The experimental data is presented in Appendix E.

Conclusions Based on Bioassay

1. An as received residual fuel oil can be mutagenic or non-mutagenic.
2. Our column pre-separation technique can separate an oil into mutagenic and non-mutagenic fractions.
   a. Our fraction #3 is generally most mutagenic of the seven fractions obtained.
   b. An oil that is non-mutagenic *in toto* can yield mutagenic fractions.

3. DMSO partitioning of an oil appears to separate it into a mutagenic and a non-mutagenic fraction.
    a. This separation is on different principles than that of our column chromatography pre-separation.
    b. The basic technique of DMSO partitioning can concentrate mutagens and "eliminate" much of the matrix. [Residual fuel oil #16 is 15% DMSO soluble, 85% insoluble.]
4. Since DMSO does seem to separate mutagens from non-mutagens, it can be an important tool for investigating bio-hazards of other forms of "soluble" energy such as Syn Fuels and Shale Oils.
5. Since a DMSO extract contains so little aliphatic organics, the solvent should be very useful in studying crude oils and refined products.
6. DMSO partitioning followed by our column chromatography pre-separation would offer maximum information on sample mutagenicity.

ORGANIC ANALYSES

Sample Fluorescence Effects

It was discovered during this contract work that mutagenicity of our column separation fractions appeared to be associated with orange fluorescence of the fraction under ultraviolet irradiation. An orange fluorescence appears unusual in terms of literature data for compounds and classes of compounds, although the literature work is based on samples in solution rather than as deposits. The "Handbook of Chemistry and Physics", by the CRC Press, no longer contains a listing,but the 41st (1959-60) edition has a list (p. 3004, ff.) containing about 350 organic materials. Of these only 2, anthraquinone and phycoerythrin, are quoted as fluorescing orange. Only an additional 45 are quoted as fluorescing yellow, brown or red showing the relative rarity of fluorescence in this region of the spectrum. Twenty of the organic compounds that, by their nature, could conceivably be in a residual oil are shown in Table 25. Most of the entries are ketones derived from polycyclic aromatic hydrocarbons which is important information. However, the data also suggest that C=O on an aliphatic backbone can also cause such fluorescence. (Due to some changes in chemical nomenclature since the data of Table 50 was published, some structures given may not be exact. These are noted with question marks.) Generally, increase in the number of condensed aromatic rings in a molecule will produce a progression toward red in fluorescence with heteroatoms such as sulfur and oxygen acting to quench any fluorescence.

Various samples of oils and fractions were examined under ultraviolet (UV) light at 366 nm. This was done by depositing portions of the sample from solution onto small discs of filter-paper and "drying". The results of this examination follow. Photos of some of the more striking results are included in this report, as referenced below.

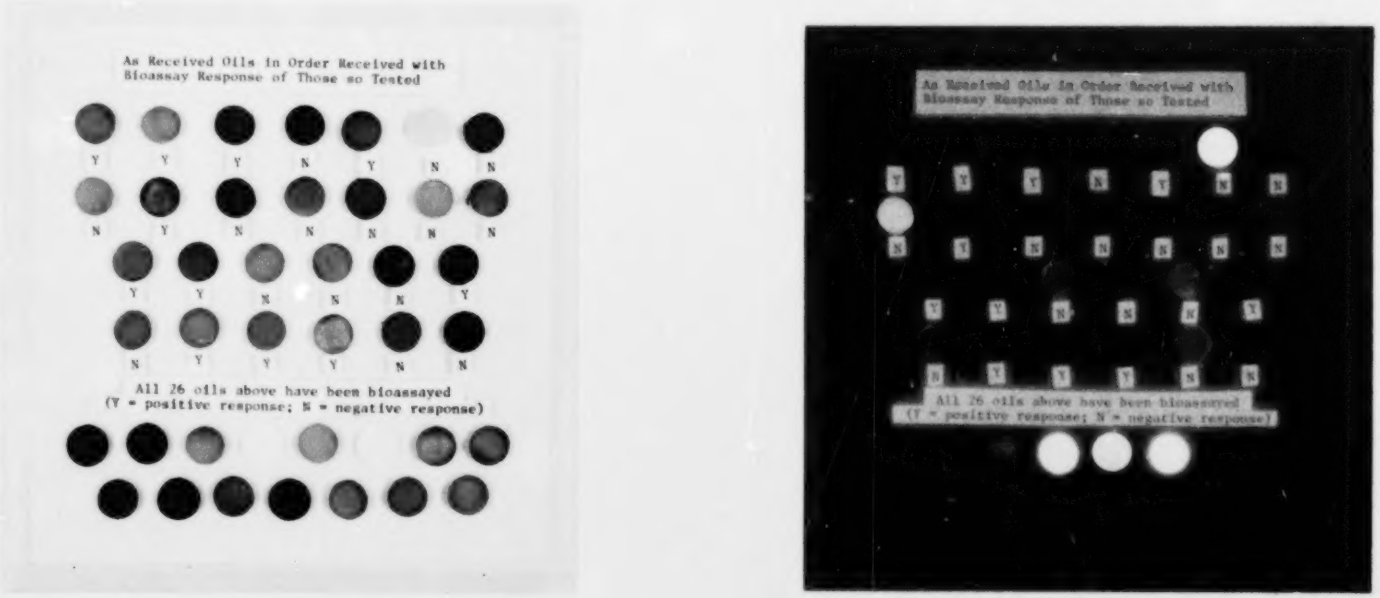
As Received Residual Fuel Oils [re: Figure 51]

Thirty-six of the as-received oil samples appeared some shade of brown under UV light. One sample was deep yellow, one was white, two were blue and one was non-fluorescent black. The results showed that these as-received oils are not ordinarily differentiated by this means. The "non-fluorescent black" sample would be the most interesting non-conforming sample. The two samples showing blue fluorescence are of the consistency of "vaseline" and of a much lighter than normal color leading to the question of how representative of a residual oil they may in fact be.

Column Chromatography Pre-separation Fractions [re: Figure 52]

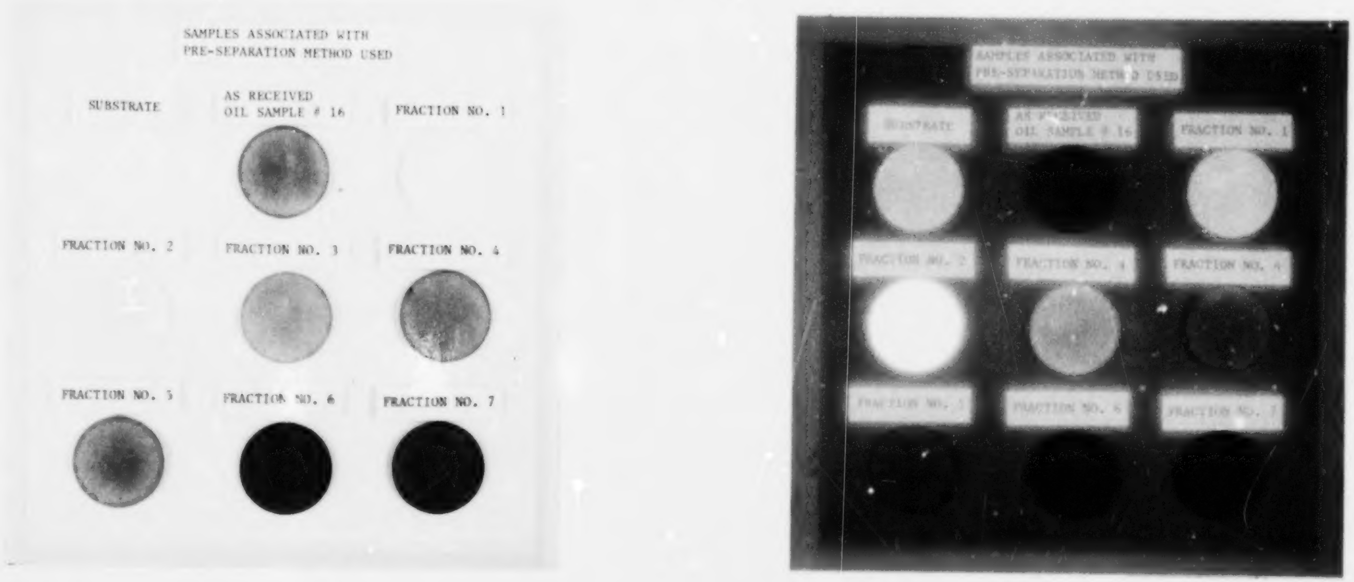
Based on W oil #16 column chromatography pre-separation fractions the fluorescence observed was;

Fraction	#1	colorless
	2	yellowish white
	3	yellow orange


As Received Oils in Order Received with
Bioassay Response of Those so Tested
Y Y Y N Y N N
N Y N N N N N
Y Y N N N Y
N Y Y Y N N
All 26 oils above have been bioassayed
(Y = positive response; N = negative response)
As Received Oils in Order Received with
Bioassay Response of Those so Tested
Y Y Y N Y N N
N Y N N N N N
Y Y N N N Y
N Y Y Y N N
All 26 oils above have been bioassayed
(Y = positive response; N = negative response)

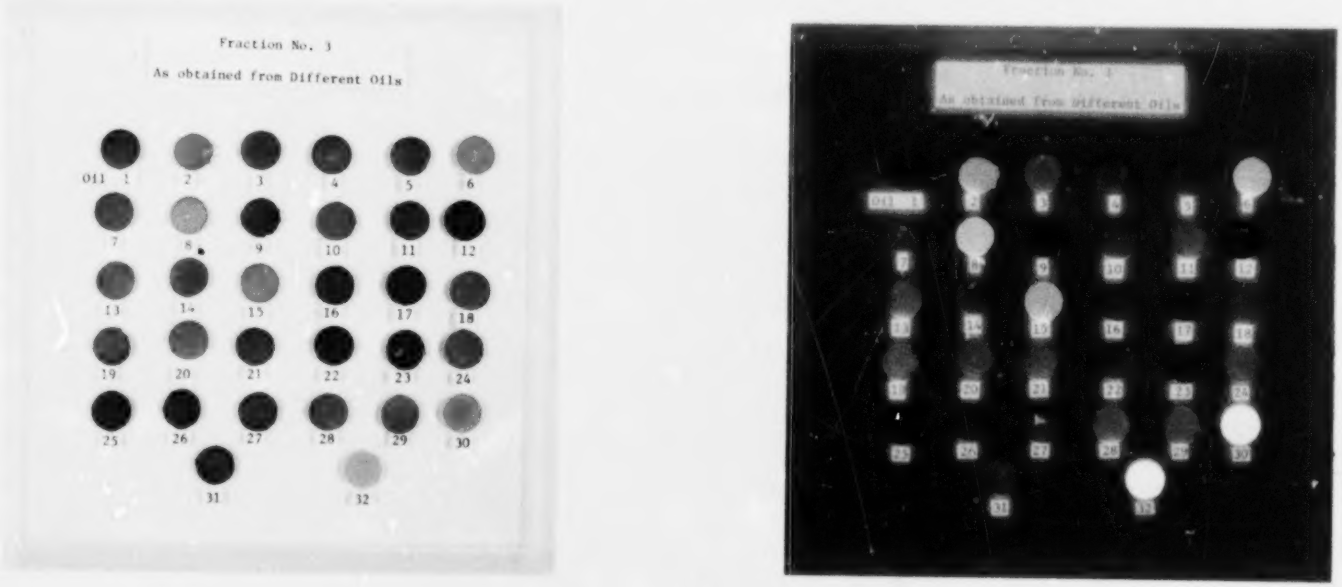
Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 51. Residual Oil Samples Under Natural Light (Left) and Ultraviolet Irradiation (Right) - As Received Residual Fuel Oil Samples


SAMPLES ASSOCIATED WITH
PRE-SEPARATION METHOD USED
SUBSTRATE
AS RECEIVED
OIL SAMPLE # 16
FRACTION NO. 1
FRACTION NO. 2
FRACTION NO. 3
FRACTION NO. 4
FRACTION NO. 5
FRACTION NO. 6
FRACTION NO. 7

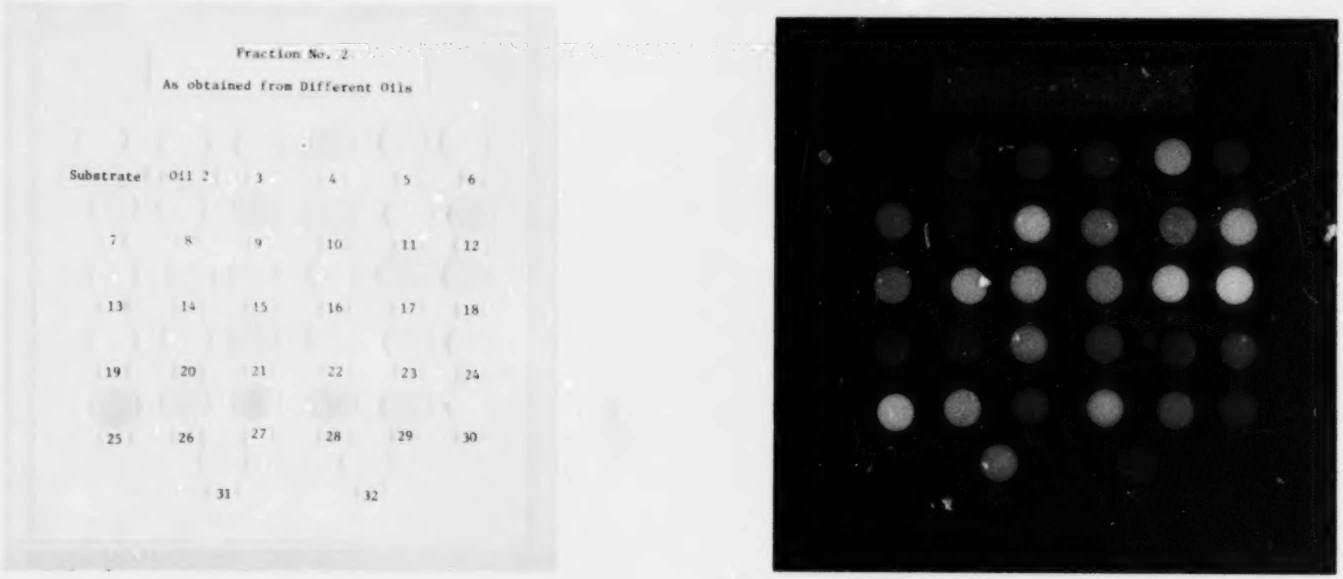
Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 52. Residual Fuel Oil Samples Under Natural Light (Left) and Ultraviolet Irradiation (Right) - Column Chromatography Fractions of Method Adopted


Fraction No. 3
As obtained from Different Oils
Oil 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

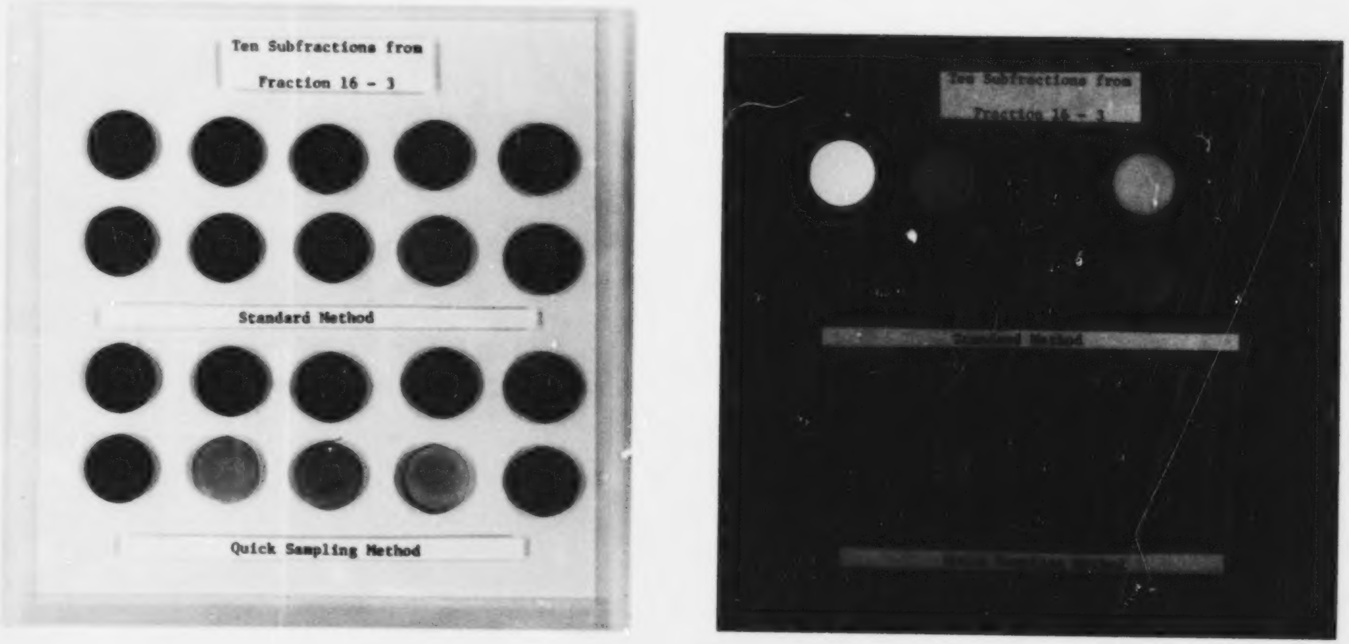
Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 53. Residual Fuel Oil Samples Under Natural Light (Left) and Ultraviolet Irradiation (Right) - "Fraction #3" from Thirty-Two Oils


Fraction No. 2
As obtained from Different Oils
Substrate
Oil 2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

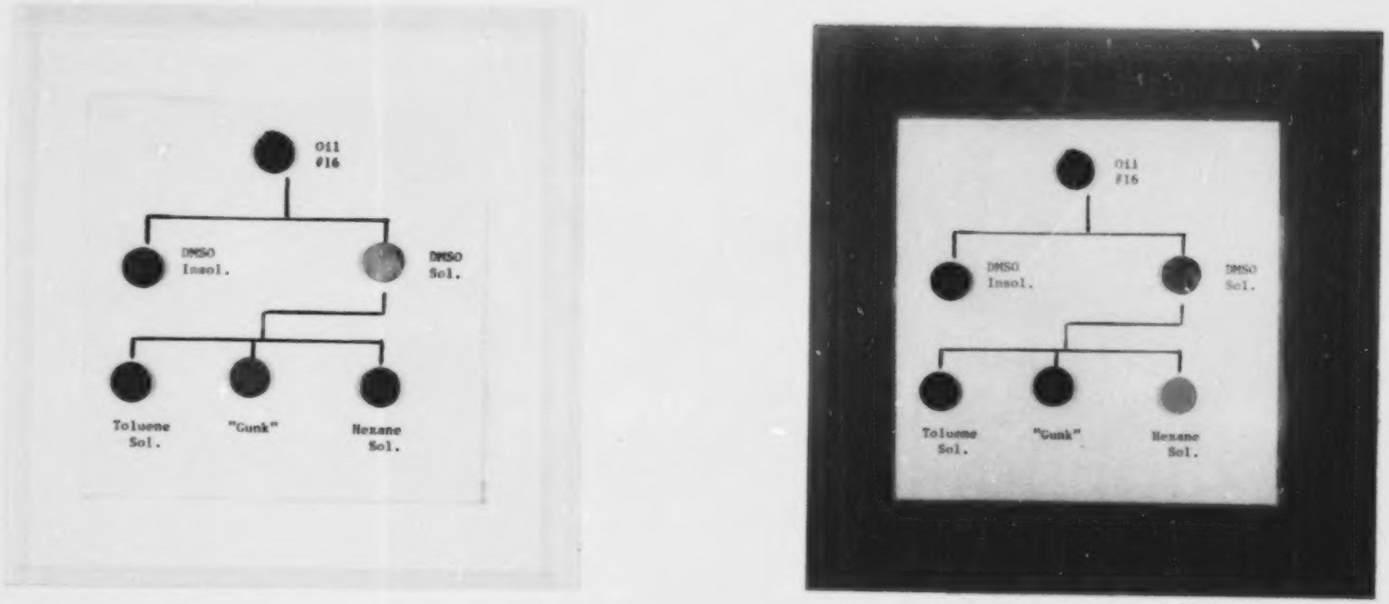
Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 54. Residual Fuel Oil Samples Under Natural Light (Left) and Ultraviolet Irradiation (Right) - "Fraction #2" from Thirty-One Oils


Ten Subfractions from
Fraction 16 - 3
Standard Method
Quick Sampling Method
Ten Subfractions from
Fraction 16 - 3
Standard Method

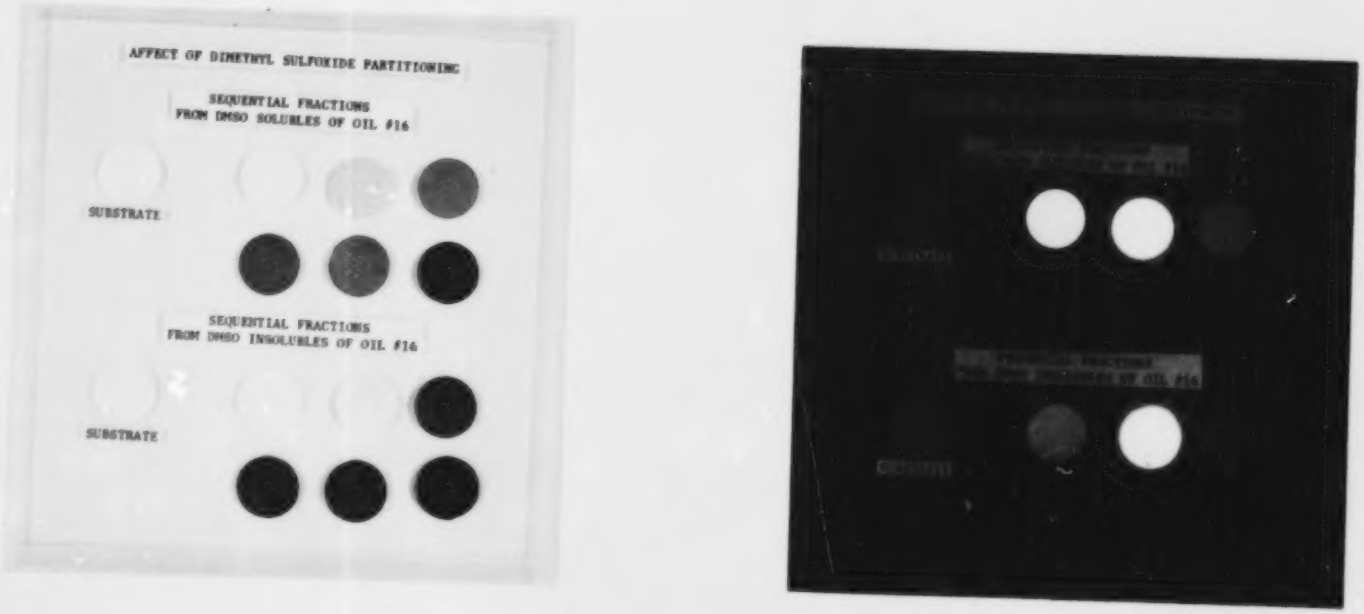
Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 55. Residual Fuel Oil Samples Under Natural Light (Left) and Ultraviolet Irradiation (Right) - Ten Sub-Fractions of a Fraction #3


Oil #16
DMSO Insol.
DMSO Sol.
Toluene Sol.
"Gunk"
Hexane Sol.
Oil #16
DMSO Insol.
DMSO Sol.
Toluene Sol.
"Gunk"
Hexane Sol.

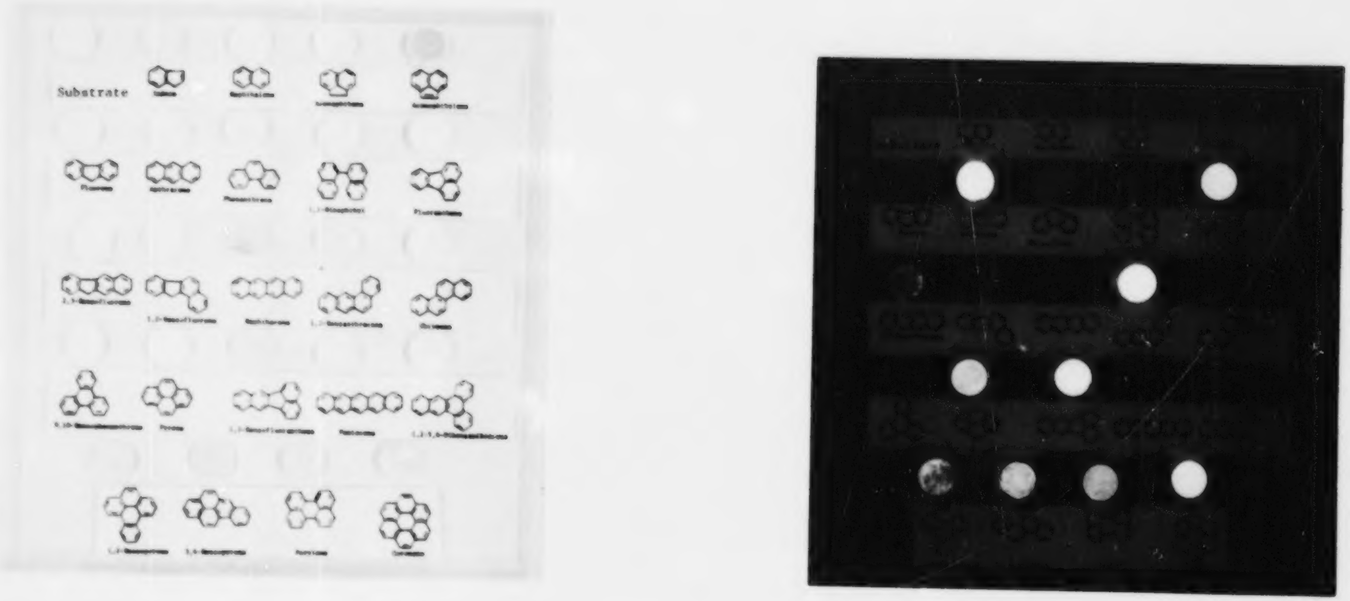
Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 56. Residual Fuel Oil Samples Under Natural Light (Left) and Ultraviolet Irradiation (Right) - A DMSO Partitioned Oil


AFFECT OF DIMETHYL SULFOXIDE PARTITIONING
SEQUENTIAL FRACTIONS
FROM DMSO SOLUBLES OF OIL #16
SUBSTRATE
SEQUENTIAL FRACTIONS
FROM DMSO INSOLUBLES OF OIL #16
SUBSTRATE

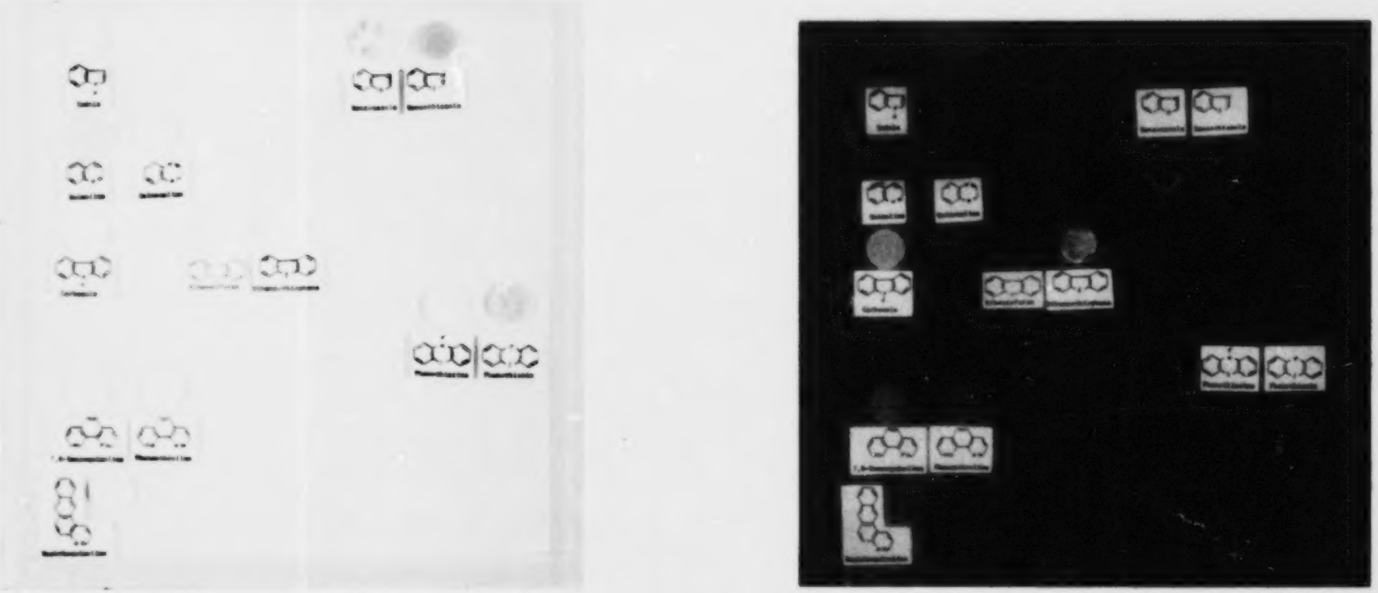
Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 57. Residual Fuel Oil Samples Under Natural Light (Left) and Ultraviolet Irradiation (Right) - Column Chromatography Fractions of DMSO Solubles and Insolubles



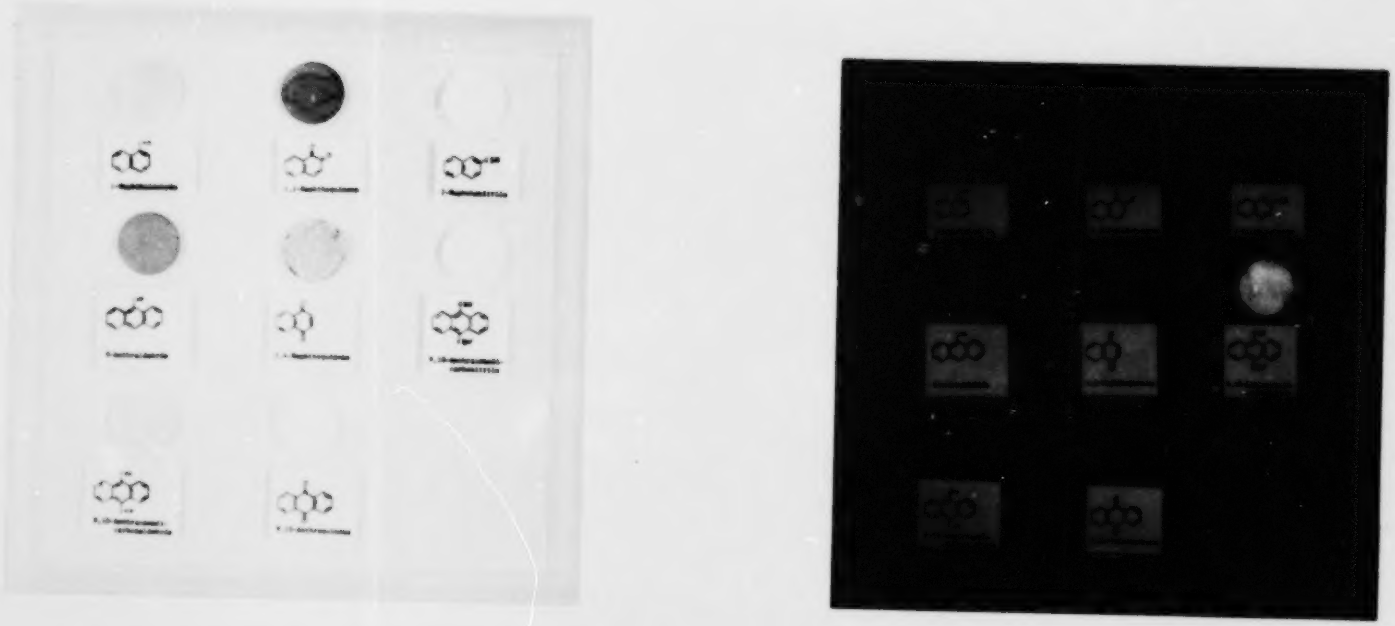
Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 58. Reference Materials Under Natural Light (Left) and Ultraviolet Irradiation (Right) - Polycyclic Aromatics



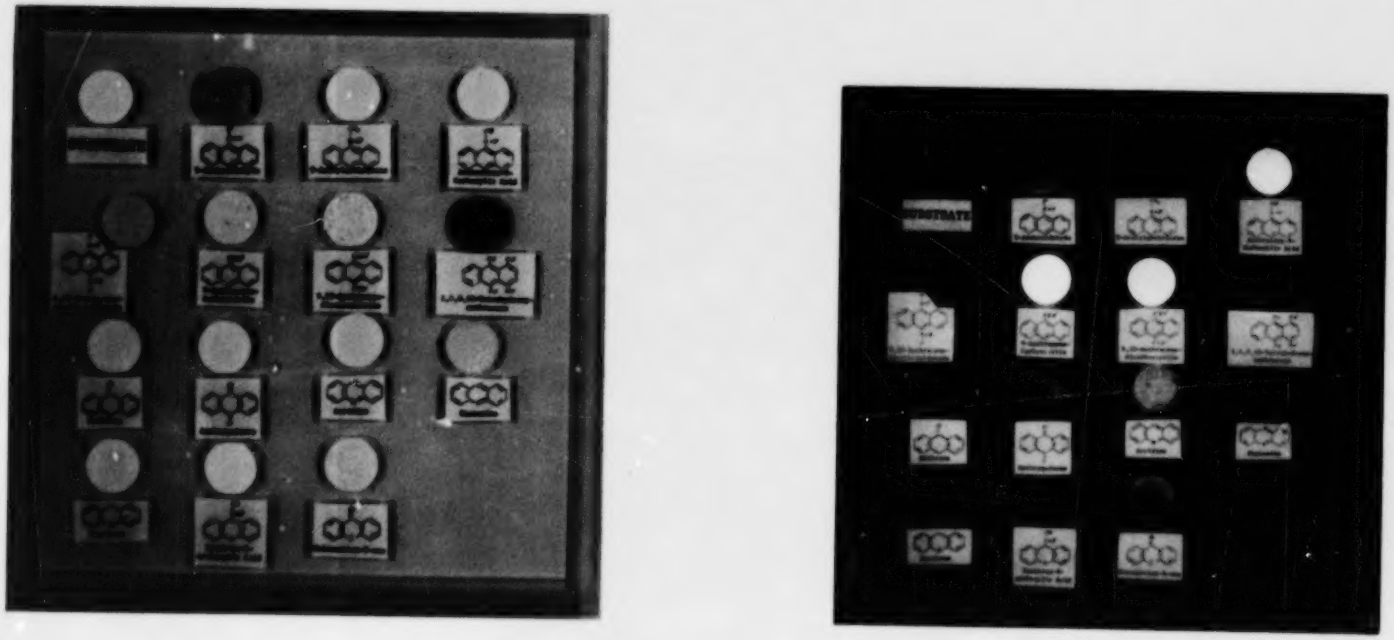
Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 59. Reference Materials Under Natural Light (Left) and Ultraviolet Irradiation (Right) - Heterocyclic Aromatics



Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 60. Reference Materials Under Natural Light (Left) and Ultraviolet Irradiation (Right) - Carbonyl Compounds of Polycyclic Aromatics



Note: The text contains color descriptions of the spots as interpreted by an observer. These may not always agree with what the color camera sees.

Figure 61. Reference Materials Under Natural Light (Left) and Ultraviolet Irradiation - Substituted Anthracenes

TABLE 25

ORGANIC MATERIALS THAT FLUORESCE YELLOW, ORANGE, BROWN OR RED

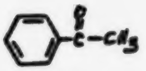
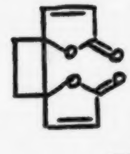
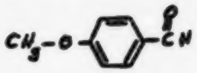
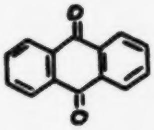
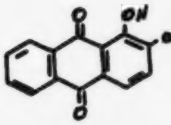
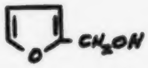
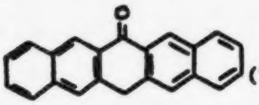
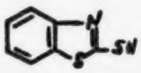
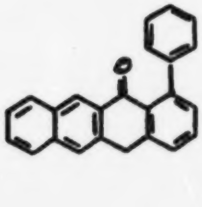
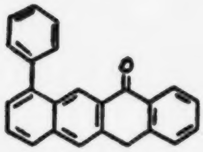
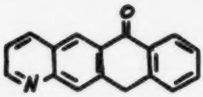
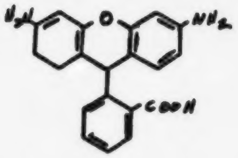
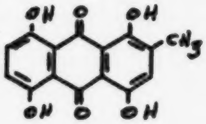
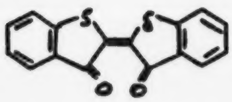
Substance	Type of Fluorescence	Structure	Molecular Weight	Boiling Point
Acetophenone	brownish		120	202°C
Anemonin	yellowish		192	
Anisaldehyde	pale brown		136	247°C
Anthraquinone	orange		208	286°C (sub.)
1,2-Dihydroxy-anthraquinone	yellow		240	430°C
Furfuryl Alcohol	deep yellow		98	171°C
Isodibenzanthrone	brown	 (?)	294	
2-Mercaptobenzothiazole	reddish-brown		167	
Myristaldehyde	brownish-yellow	$CH_3(CH_2)_{12}CHO$	212	
Myristone	yellowish	$CH_3(CH_2)_{11}COCH_3$ (?)	212	
1-Phenyl-benzanthrone	orange-red	 (?)	320	

Table 25 (Cont)

Substance	Type of Fluorescence	Structure	Molecular Weight	Boiling Point
10-Phenyl-benzanthrone	golden yellow	 (?)	320	
Protoporphyrin	red	(?)		
Pyridanthrone	yellow	 (?)	245	
Rhodacine	red	$C_{30}H_{20}$ (a pyrolytic product from acenaphthene)		
Rhodamine	red-orange		332	
1,4,5,8-tetrahydroxy-2-methyl-anthraquinone	red		286	
Thioindigo	red-yellow		296	(sub.)

Fraction #4	brownish orange
5	brownish orange
6	black
7	deep brown

The above differences in fluorescence confirm that the column chromatography pre-treatment is effective for preliminary separation of oil samples prior to further analyses.

"Fraction #3" from Thirty-two Residual Fuel Oils [re: Figure 53]

In general, fluorescence of all "Fraction #3" samples was yellow-orange or orange. Two samples were light yellow (corresponding to the vaseline-like oils discussed above) and one was brown under the UV light. It should be remembered that in a fraction #3 we are dealing with the most likely residence for mutagenicity of an oil. Therefore, this consistent orange fluorescence is important and can lead to classification and perhaps identification of materials that most frequently (more potently?) lead to environmental problems.

"Fraction #2" from Thirty-One Residual Fuel Oils [re: Figure 54]

A fraction #2 from an oil would contain the polycyclic aromatic hydrocarbons (PAH) which are usually associated with mutagenicity in the literature. "Fraction #2" as obtained from 31 residual fuel oils were examined by UV. In all cases, fluorescence was light greenish yellow, light blue, or deep violet blue. This result confirms the sharp differentiation that exists between our pre-treatment fractions #2 and #3.

Ten Sub-Fractions of Oil #16, Fraction #3 [re: Figure 55]

These 10 fractions fluoresced from orange yellow through orange red as in Table 26. This suggests that while there is some separation among the 10 fractions, it may not be very sharp. Our pre-treatment column, designed to separate chemical classes, may have to be redesigned in order to better separate individual compounds.

TABLE 26

DATA ON TEN SUB-FRACTIONS OF OIL FRACTION 16-3

Sub-fraction	% of Original Sample	Fluorescence under Long Wave UV	Comments
16-3-1	1.74	Orange yellow	The sharpest change in fluorescence is between 16-3-1 and 16-3-2. However, the stepwise change 16-3-2 to 16-3-10 is distinct.
16-3-2	4.84	Orange	
16-3-3	3.06	↓	
16-3-4	2.15	↓	
16-3-5	1.15	↓	
16-3-6	0.65	↓	
16-3-7	0.15	↓	
16-3-8	0.43	↓	
16-3-9	0.02	↓	
16-3-10	0.08	Orange red	
TOTAL	14.3		
PREVIOUSLY*	17.8		

* Original determination, 31 August 1978

At this point an attempt was made to shorten our column pre-separation method by casting sample directly onto the top of the column rather than pre-coating, pre-heating on 25 grams of column material. The ten subfractions collected, "corresponding" to the above ten fractions appeared black (quenching?) under ultraviolet irradiation (see Figure 55), indicating poorer separation or a different process of separation. There was no purpose to pursuing this at this point.

Further Sub-fractionation of a Fraction #3

A 5/8" ID glass tube was loaded with 30 inches of our column material. About 4 grams (3.9998 g) of conditioned oil #34 in 25 grams fluorisil/Na_2SO_4 was placed atop and the column developed with hexane and hexane/15% toluene. Methylene chloride was then passed through the column and 30-5 ml eluant cuts and 24-50 ml cuts collected. The fluorescence of the deposits from these solutions were as follows:

Five-milliter cuts
#1 = deep yellow
#2 = orange yellow
#3 through #30 = orange and deep orange with no pattern (intensity variation may be a factor of quantity of material available in a cut).

Fifty milliliter cuts
All cuts = orange or deep orange, except cut #18 which was orange brown.

Once again the column may not be acting as a good separator of individual compounds. The dispersion/conditioning of 4 grams of sample on column material, so effective in chemical class separation, probably produces too diffuse a "front" for compound separation.

Fractions from DMSO Partitioning of an Oil [re: Figure 56]

Oil sample #16 was partitioned with DMSO as illustrated in Figure 62. The figure includes infra-red analysis data, which will be discussed

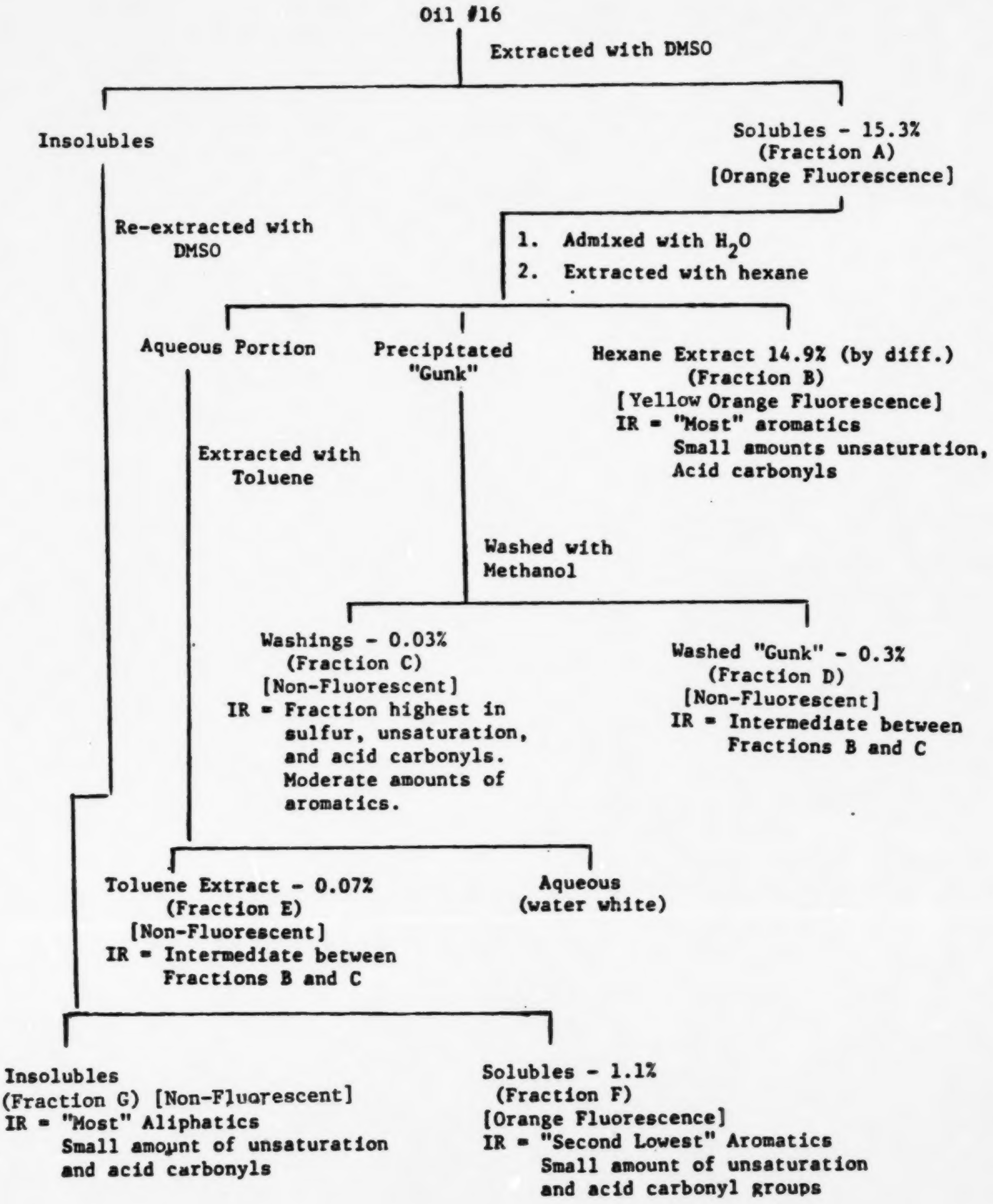

Oil #16
Extracted with DMSO
Insolubles
Solubles - 15.3%
(Fraction A)
[Orange Fluorescence]
Re-extracted with DMSO
1. Admixed with H_2O
2. Extracted with hexane
Aqueous Portion
Precipitated "Gunk"
Hexane Extract 14.9% (by diff.)
(Fraction B)
[Yellow Orange Fluorescence]
IR = "Most" aromatics
Small amounts unsaturation,
Acid carbonyls
Extracted with Toluene
Washed with Methanol
Washings - 0.03%
(Fraction C)
[Non-Fluorescent]
IR = Fraction highest in sulfur, unsaturation, and acid carbonyls. Moderate amounts of aromatics.
Washed "Gunk" - 0.3%
(Fraction D)
[Non-Fluorescent]
IR = Intermediate between Fractions B and C
Toluene Extract - 0.07%
(Fraction E)
[Non-Fluorescent]
IR = Intermediate between Fractions B and C
Aqueous
(water white)
Insolubles
(Fraction G) [Non-Fluorescent]
IR = "Most" Aliphatics
Small amount of unsaturation and acid carbonyls
Solubles - 1.1%
(Fraction F)
[Orange Fluorescence]
IR = "Second Lowest" Aromatics
Small amount of unsaturation and acid carbonyl groups

Figure 62. Solvent Partitioning of Oil #16

later, and a listing of fraction fluorescence effects. For convenience the latter are also summarized here:

- DMSO - insolubles = non-fluorescent (dark brown)
- DMSO solubles = orange
    - Hexane solubles = yellow orange
    - "Washed Gunk" = non-fluorescent (black)
    - Toluene solubles = non-fluorescent (dark brown)

The results with the DMSO treatment appear important because they could lead to by-passing the present column pre-separation or at least to concentrating (by a factor of up to 7), if oil #16 is an example, the "active" portion of a sample as introduced atop the column. The instances of orange fluorescence are also important in demonstrating residence of affect in the sample rather than in an artifact - this oil has seen neither our pre-treatment column nor our supply of methylene chloride nor any of the first 2 column eluting systems. The non-fluorescence of the "washed gunk" is also important. This material was found to be mutagenic by Ames test as performed by Westinghouse. This is the one and only time a completely non-fluorescent (quenced?) sample was found to be mutagenic.

Fluorescence of Column Chromatography Fractions from DMSO Solubles and Insolubles [re: Figure 57]

There was very little difference in fluorescence between column chromatography fractions from the DMSO soluble and DMSO insoluble portions of Oil Sample 16:

	Fluorescence	
Fraction	DMSO Solubles	DMSO Insolubles
1	blue white	dull blue
2	light yellow	yellowish white
3	orange	red orange
4	red brown	red brown
5	red brown	orange brown
6	black brown	black

insufficient material for test

Concepts that would bear keeping in mind for checking with a realistic number of samples are:

1. While "most potent" mutagenicity resides in a fraction of orange fluorescence, not all fractions of orange fluorescence are mutagenic. Fluorescence can reside in very low levels of material occurrence so that normal carry-over between DMSO soluble/insoluble can affect subsequent fluorescence of column chromatography fractions.
2. Orange fluorescence may originate with functional groupings rather than within base molecules - DMSO solubles being basically aromatic in nature and DMSO insolubles being basically aliphatic.

Fluorescence of Model Compounds

In addition to the above work with samples, different classes of organic polycyclics were subjected to UV examination as deposits on filter paper discs:

Polycyclic aromatics: [re: Figure 58]

The following were UV irradiated with the results as shown:

Indene	non-fluor.	1,2-Benzanthracene	yellow green
Naphthalene	non-fluor.	Chrysene	violet blue
Acenaphthene	non-fluor.	9,10-Benzophenanthrene	violet blue
Acenaphthylene	non-fluor.	Pyrene	blue white
Fluorene	blue	1,2-Benzofluoranthene	blue
Anthracene	yellow	Pentacene	brown
Phenanthrene	blue	1,2:5,6-Dibenzanthracene	violet blue
1,1-Binaphthyl	violet blue	1,2-Benzopyrene	blue
Fluoranthene	blue white	3,4-Benzopyrene	green bluw
2,3-Benzofluorene	violet blue	Perylene	orange
1,2-Benzofluorene	violet blue	Coronene	green yellow
Naphthacene	reddish		

It is seen that these are "fraction 2" type materials in terms of fluorescence, including α-benzopyrene. Perylene is the only material that appears to fluoresce orange (impurity?) but its behavior on our column has not been determined.

Heterocyclic Organic Materials: [re: Figure 59]

Polycyclic heterocycles were examined in terms of one nitrogen, two nitrogens, one oxygen, one sulfur, nitrogen-oxygen, nitrogen-sulfur, and oxygen-sulfur analogues of polycyclic hydrocarbons. The results:

Indole	non-fluor.	Dibenzofuran	blue-gray
Benzoxazole	non-fluor.	Dibenzothiophene	violet
Benzothiazole	non-fluor.	Phenothiazine	non-fluor.
Quinoline	non-fluor.	Phenothioxin	non-fluor.
Quinoxaline	non-fluor.	7,8-Benzoquinoline	violet blue
Carbazole	blue	Phenanthroline	non-fluor.
		Naphthoquinoline	blue

Based on the above, these materials are not represented in the orange fluorescence in Fraction 3 from an oil.

Carbonyl Compounds of Polycyclic Aromatics: [re: Figure 60]

Fluorescence results with these types of compounds were as follows:

1-Naphthaldehyde	non-fluor. (gray black)
1,2-Naphthaquinone	non-fluor. (dark brown)
1,4-Naphthaquinone	non-fluor. (black)
2-Naphthonitrile	non-fluor.
9-Anthraldehyde	yellow
9,10-Anthracenedicarboxaldehyde	non-fluor.
9,10-Anthraquinone	blue
9,10-Anthracenedicarbonitrile	yellow

It is possible that 9-anthraldehyde and 9,10-anthracenedicarbonitrile could be present in the fluorescent materials of a Fraction 3. Their behavior on our column would have to be investigated. It is unfortunate that model carbonyl compounds of more highly condensed aromatics are not available since fluorescence would be shifted toward the red. It is interesting to note that the literature, as quoted above, lists an orange fluorescence for anthraquinone while we note a blue fluorescence. No data is given in the literature reference on how the test sample was prepared or what solvent, if any, was used.

Substituted Anthracenes: (re: Figure 61]

Since two of the anthracene-based compounds above showed near-orange fluorescence, available model compounds with anthracene-type backbones were examined under UV:

9-anthraldehyde	yellow
9-acetyl anthracene	gray brown
Anthracene-9-carboxylic Acid	yellow
9,10-Anthracenedicarboxaldehyde	non-fluor. (brown)
9-Anthracenecarbonitrile	yellow
9,10-Anthracenedicarbonitrile	yellow
1,4,9,10-Tetrahydroxanthracene	non-fluor. (black brown)
Anthrone	non-fluor. (dark gray)
Anthraquinone	blue
Acridine	non-fluor. (gray brown)
Phenanzine	non-fluor. (gray brown)
Xanthene	non-fluor. (gray)
Xanthene-9-carboxylic Acid	non-fluor. (gray)
Thioxanthen-9-one	yellow gray

The materials fluorescing yellow above may be present in our "active" fractions.

Analyses by Gas Chromatography/Mass Spectroscopy

With the amount of work to be accomplished only a limited number of chromatographic columns were evaluated for separation of the residual fuel oil into its components. Only one packed column (1/3", with Dexsil 300) was evaluated - chosen for its rather high upper temperature limit (approx. 350°C). It was eliminated from use due to its rather poor resolution with these complex samples. SCOT columns were chosen over capillary columns because of their somewhat greater thru-put. It was felt that capillary columns might miss some of the minor sample components because of their more limited sample capacity. OV101 packing material was chosen over several others for reasons that are best explained in a later paragraph under another context.

Criteria for Evaluation of Data

Qualitative Analyses

It does appear that a trained analyst will ordinarily do a better job of identifying a mass spectrum than the GC/MS data system. Figure 63 is a spectrum of unknown component from a Fraction 7 of an oil. The trained analyst would think "phthalate ester". What the computer search of its library "thinks" is shown in Figures 64 and 65. Mass spectral examination is ultimately based on human interpretation in this work.

A serious problem in qualitative mass spectral analyses of the samples is the lack of appropriate reference spectra for identifying unknowns. This becomes increasingly critical with increasing polarity of sample fractions.

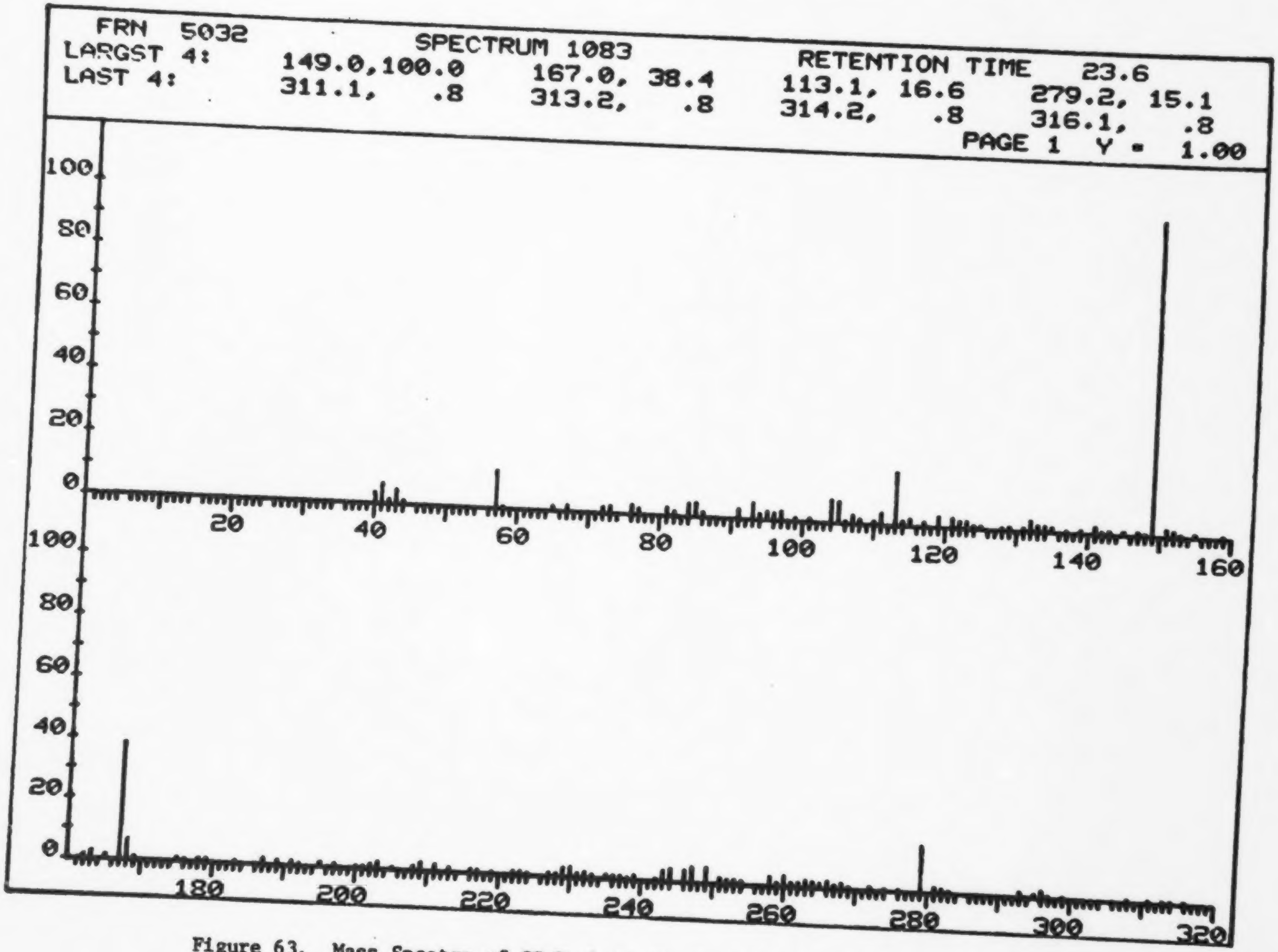


Figure 63. Mass Spectra of GC Peak in Sample 1-7 at a 23.6 Retention Time

```
LIBRARIES FOR SEARCH? N
LOW AND HIGH MOLECULAR WEIGHT LIMITS?     [    1.0_ 1000.0] 135,300
USE DEFAULT SEARCH OPTIONS?               Y
WORKING...
 FRN  5032 SPECTRUM  1083, RET.TIME:  23.57     147 PEAKS, SIG= 104
 LIBRARY 3000  14297 SPECTRA SEARCHED,    10 HIT(S)       (MAX K=  22.7)

 .7093 * 1,1'-Binaphthalene, 2,2'-dimethyl- (9CI)
         LSN= 1096 (NBS  16582) MW= 282  C22H18
         FRN= 3011 CAS:  0032834847  EPA:  0000040715   RET. TIME=    .00
         MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  250
          16.1   8   3%     .0   0   0%     .0   2   8%  MULTIPLIER=   .26

 .7093   2,4-Hexadienedioic acid, 3,4-diethyl-, dimethyl ester, (E,Z)
         - (8CI)
         LSN= 2011 (NBS  12739) MW= 226  C12H1804
         FRN= 3008 CAS:  0031545752  EPA:  0000010786   RET. TIME=    .00
         MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  918
          16.1   9  14%     .0   0   0%     .0   0   0%  MULTIPLIER=   .37

 .6916 * Naphthalene, 1-(2-naphthalenylmethyl)- (9CI)
         LSN=  141 (NBS  15627) MW= 268  C21H16
         FRN= 3011 CAS:  0000611483  EPA:  0000039316   RET. TIME=    .00
         MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  883
          15.7   8   3%     .0   0   0%     .0   2  10%  MULTIPLIER=   .29

 .6784 + 2-Oxazolidinone, 4-phenyl-5-p-tolyl-, trans- (8CI)
         LSN= 3926 (NBS  14654) MW= 253  C16H15NO2
         FRN= 3008 CAS:  0032461317  EPA:  0000039684   RET. TIME=    .00
         MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  425
          15.4   9   7%     .0   0   0%     .0   1   7%  MULTIPLIER=   .36

 .6652 + 2H-1,2-Oxazine, tetrahydro-2-methyl-6-p-tolyl- (8CI)
         LSN= 3827 (NBS   9494) MW= 191  C12H17NO
```

Figure 64. NBS Library/Computer Identification of the Spectra in Figure 7

```
         FRN= 3005 CAS:  0015769901  EPA:  0000008280
         MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  RET. TIME=   .00
          15.2   9   5%    .0   0   0%    .0   1  18%  QUAL INDEX=  391
                                                       MULTIPLIER=  .81
.6520 * 1,1'-Biphenyl, 2-phenoxy- (9CI)
         LSN= 3417 (NBS  14145) MW= 246  C18H14O
         FRN= 3008 CAS:  0006738041  EPA:  0000011904  RET. TIME=   .00
         MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  974
          14.8   8   3%    .0   0   0%    .0   2   7%  MULTIPLIER=  .25
.6476   2,4-Hexadienedioic acid, 3,4-diethyl-, dimethyl ester, (Z,Z)
        - (8CI)
         LSN= 2010 (NBS  12738) MW= 226  C12H18O4
         FRN= 3008 CAS:  0031447548  EPA:  0000010785  RET. TIME=   .00
         MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  942
          14.8   8  13%    .0   0   0%    .0   0   0%  MULTIPLIER=  .49
.6432 * Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecah
        ydro-1,1,4a,7-tetramethyl-, [4aS-(4a.alpha.,4b.beta.,7.beta.

        ,10a.beta.)]- (9CI)
         LSN=  426 (NBS  15912) MW= 272  C20H32
         FRN= 3011 CAS:  0001686664  EPA:  0000042568  RET. TIME=   .00
         MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  918
          14.5   7   5%    .0   0   0%    .0   2  12%  MULTIPLIER=  .27
.6388 + Benzene, 1,2,4,5-tetrakis(1-methylethyl)- (9CI)
         LSN= 3387 (NBS  14115) MW= 246  C18H30
         FRN= 3008 CAS:  0000635110  EPA:  0000011884  RET. TIME=   .00
         MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  968
          14.5   8   4%    .0   0   0%    .0   1   6%  MULTIPLIER=  .31
.6300 * Estra-1,3,5,7,9,15-hexaen-17-one, 3-methoxy- (9CI)
         LSN=  900 (NBS  16386) MW= 278  C19H18O2
         FRN= 3011 CAS:  0056588535  EPA:  0000047939  RET. TIME=   .00
```

Figure 64. (Cont)

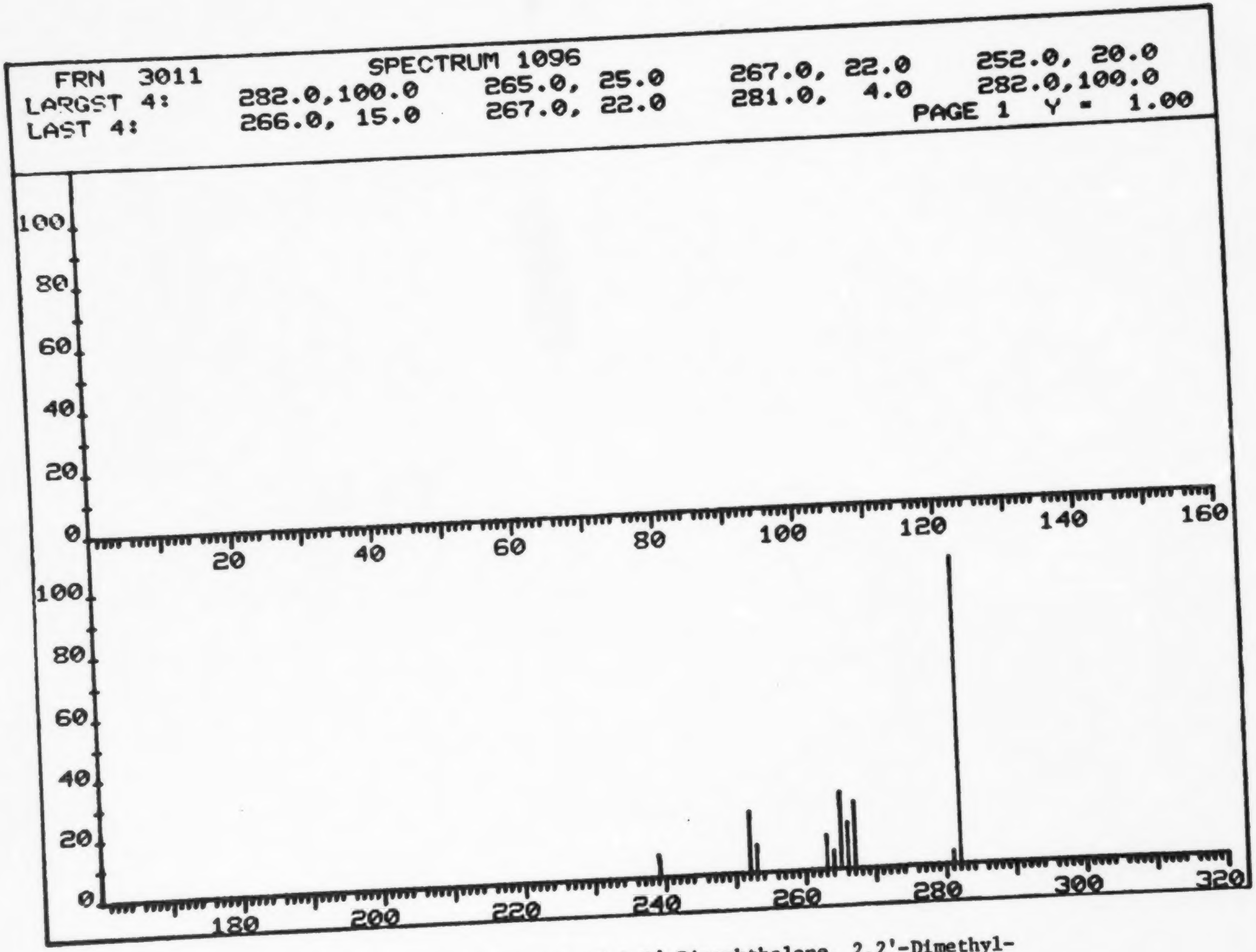


Figure 65. Mass Spectra of 1,1'-Binaphthalene, 2,2'-Dimethyl-

Quantitative Analyses

A great deal of effort has gone into attempts to quantify sample components. Unfortunately, the effort was hampered by two serious restrictions:

1. The relative amount of sample that is volatile through the GC/MS. This is generally estimated at below 15 to 20%. Occasionally, our column chromatography Fraction #2 of an oil exhibits "100%" volatility.
2. The resolution of sample components, in terms of quantitative analyses needs, is poor and degrades with increasing polarity as in going from our column chromatography Fraction 1 to Fraction 7.

The mechanism used to attempt to establish criteria for quantitative measurement was injection of a mixture of 31 model compounds of types expected in residual fuel oil:

9-Methylanthracene
1,2,3,4-Tetrahydronaphthalene
Naphthalene
2-Methylnaphthalene
1-Methylnaphthalene
2-chloronaphthalene
Biphenyl
Acenaphthalene
1,4-Dimethylnaphthalene
2,3-Dimethylnaphthalene
Azulene
Acenaphthene
Fluorene
β-Naphthol
Phthalic Anhydride
Pentacene
9,10-Dihydroanthracene
Anthracene
Phenanthrene
1,1-Binaphthyl
Terphenyl
Fluoranthene
Perylene
Pyrene
1,2-Benzofluorene
Triphenylene
Chrysene
3,4-Benzopyrene
2-Methylanthracene
1,2:5,6-Dibenzanthracene
Naphthacene

The response obtained with this synthetic sample was equivalent to about 30 million ion counts when compared to the weight of residual fuel oil sample used for analyses. This figure was then chosen as the ion count level of a total volatile sample with 30 ion counts corresponding to each ppm of occurrence.

As Received Oil Samples

Three as received oils were analyzed by GC/MS to qualify that the samples were complex enough to warrant pre-fractionation prior to introduction into GC/MS instrumentation. This case can be readily demonstrated by comparing one of these as received oils to some of the fractions prepared from it:

Sample	No. of GC Peaks (at 1% above Threshold)
As Received Oil #1	68
Fraction 1-2 of Oil #1	331
Fraction 1-3 of Oil #1	368
Fraction 1-7 of Oil #1	163

Column Chromatography Pre-Fractions

Fractions 1

Column chromatography Fraction 1 from 6 oils, samples 2 through 7, were examined to see if they contained significant quantities of material other than aliphatic hydrocarbons. The total ion chromatograms of the samples are shown in Figure 66. Analysis shows that the major peaks are due to paraffins from $C_{12}H_{26}$, at retention times about 6 minutes, to $C_{22}H_{46}$ at retention time about 20.6 minutes. The mass spectra of the remaining major peaks are clearly and cleanly aliphatic and probably encompass $C_{23}H_{48}$ (molecular weight 324) to $C_{31}H_{64}$ (molecular weight 436). Mass scanning was performed only out to 320 mass units during data acquisition. Mass spectra of the minor peaks are not as clear but they seem to be primarily aliphatic. Some C_nH_{2n} may be

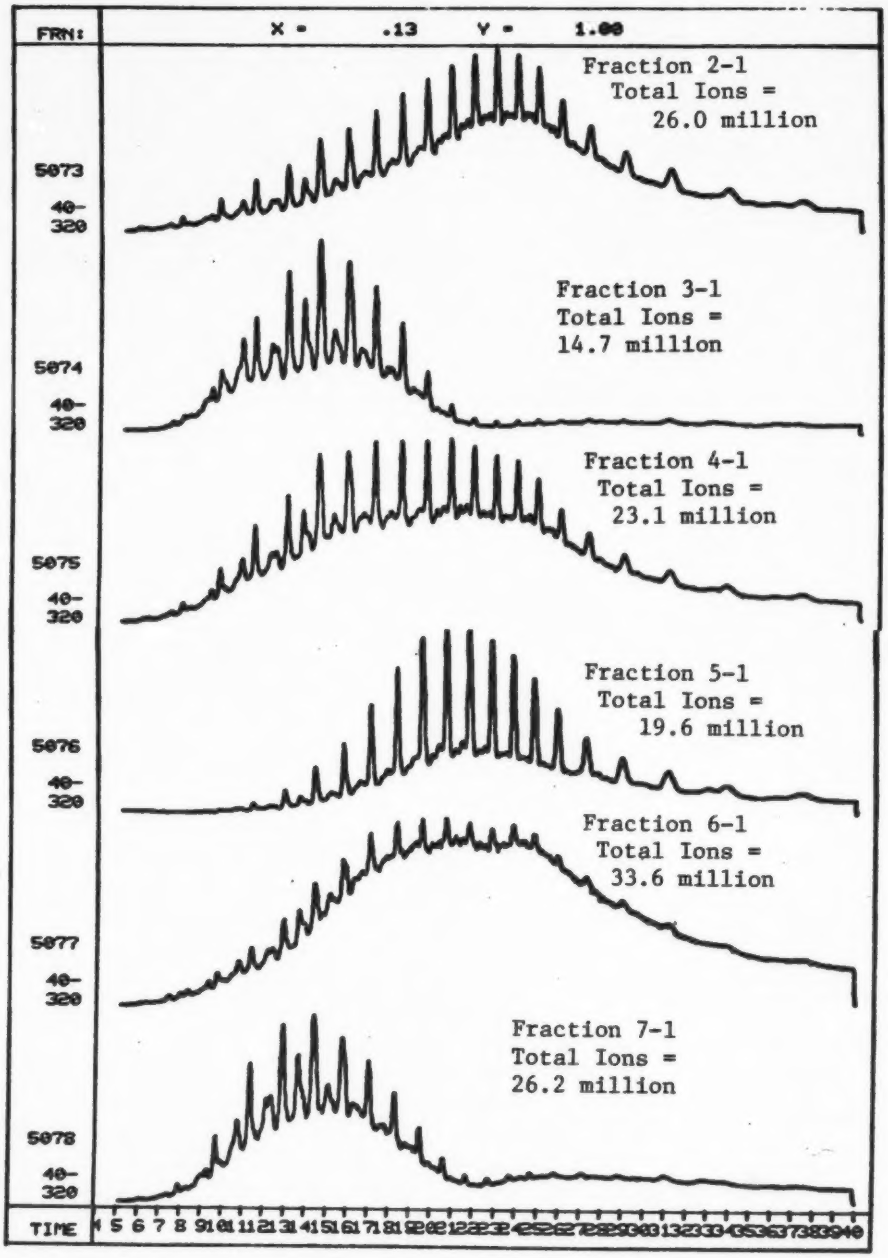
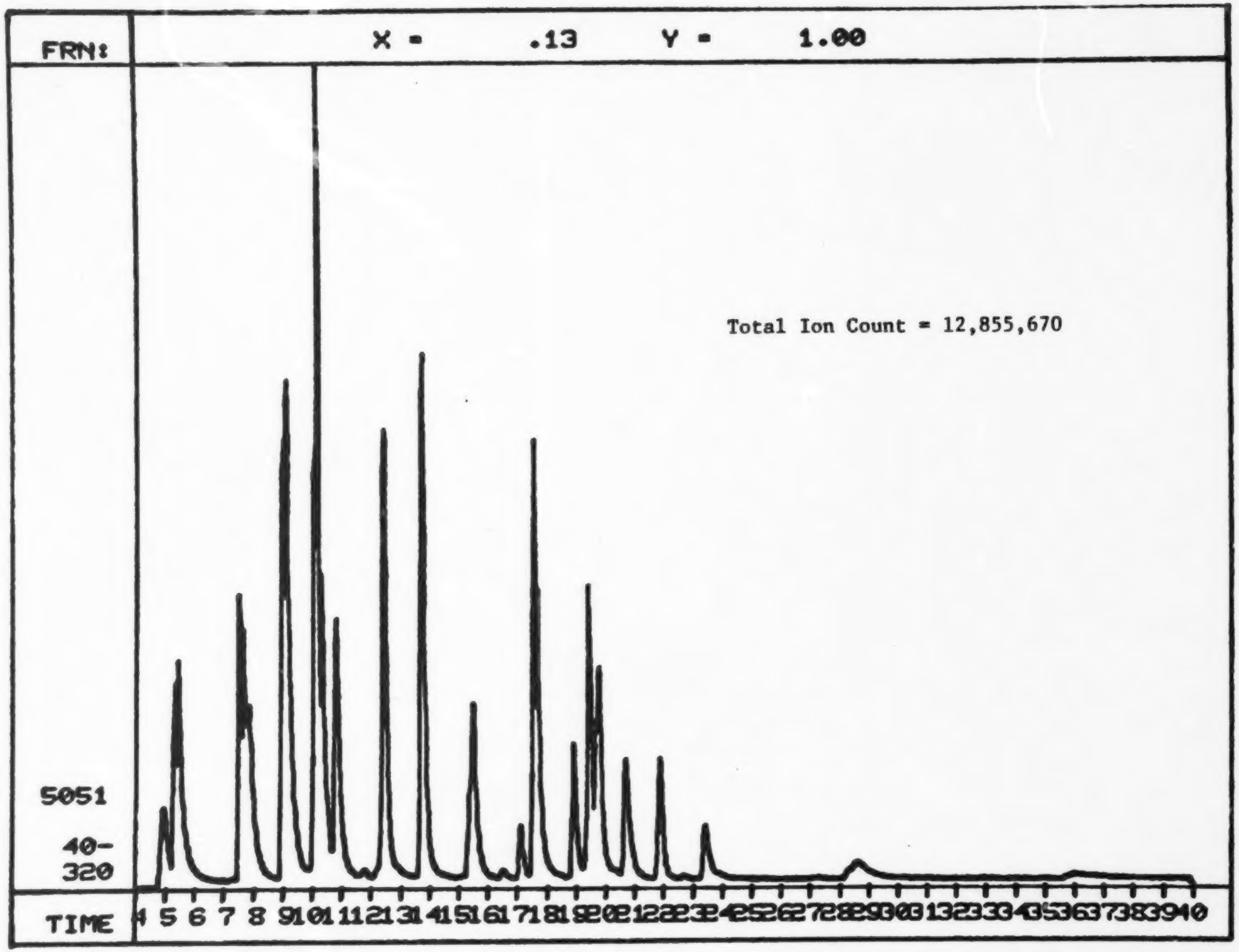


Figure 66. Total Ion Chromatograms of Fraction 1, Oils 2 through 7

indicated. There could be some aromatic hydrocarbon peaks in the data, but never where there is no "noise", which obscures the interpretation. A disappointing feature of the chromatograms of Figure 66 is the large "bulge" of unresolved material that can be seen with most samples. This bulge is not seen with the standard used for quantitation (Figure 67). This bulge, unfortunately, is seen with other fractions of these residual oils. While the bulge material is counted as part of the total "volatility" of a fraction, it is unresolved and not counted in peak areas. It is normally the case that the peaks that are found and examined are really generally a very small part of the sample being investigated. The major conclusions that would be made at this point are that GC/MS will not give a complete analysis of these samples, and that there is no alternative now to getting this analysis. The complexity of the material in a "bulge" is shown by the mass spectra of Figure 68 for sample 6-1. The data in the spectra do not appear to be particularly significant, but there is so much of it and important components could be hidden. All in all, it does not seem worthwhile to be investigating the first fraction from column chromatography pre-separation of residual oil samples.

Fractions 2 through 7

The first important point in the analysis of residual fuel oils is to have an understanding of what portion of the sample is undergoing GC/MS analysis. This is best shown through data as in Table 27. Item 3 in Table 27 is the only area where any qualitative or quantitative information will be gained in this study. The data source at 9.3% for as-received oil sample 1 may well be the highest of such an oil, while 0.6% as for oil 2 may not be unusually low. Item 4 in the table may provide an area for obtaining additional information on residual oils. It represents volatile material with the problem being sample resolution by the GC column. Based on the complexity of the samples, it may be impossible to resolve this portion of the sample by any means currently available.



Total Ion Count = 12,855,670

Figure 67. Total Ion Chromatogram of Standard Mixture

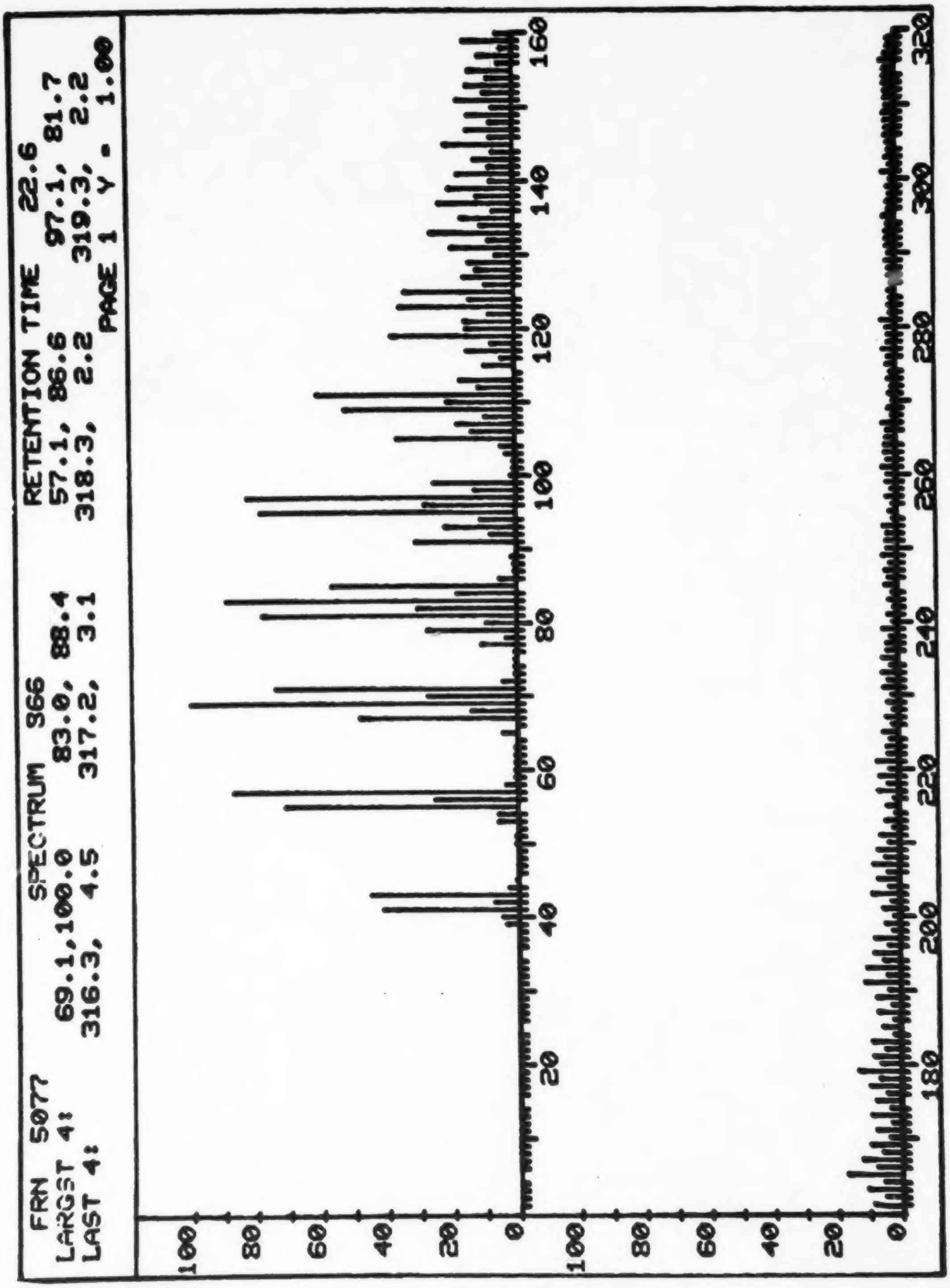


Figure 68. Mass Spectra of Unresolved ("Bulge") Material in Sample 6-1

Table 27

EXAMPLES OF SAMPLE THROUGH-PUT DURING GC/MS ANALYSES OF RESIDUAL FUEL OILS

	Sample Through-Put			
	As Percent of Injected Sample*		As Percent of As-Received Sample**	
Item	Oil #1	Oil #2	Oil #1	Oil #2
1. Injections summed, fractions 2 through 7 from pre-separation of samples.	100	100	61.7	52.3
2. Total volatiles in fractions 2 through 7 as determined by summing total ion counts for GC traces of individual fractions.	48.3	48.9	29.8	25.6
3. Sum of all ions in total volatiles "identified" in all fractions in terms of GC peaks having reasonably clean mass spectra. This portion is potentially identifiable given proper reference data on model compounds.	15.1	1.1	9.3	0.6
4. Portion of sample that is volatile but unresolved by GC column. This material would not be identifiable through any reference data.	33.1	47.8	20.5	25.0
5. Portion of sample which can probably never be analyzed by standard GC/MS.	51.7	51.1	—	—

---

*Does not include fraction 1 (aliphatics) and material not recovered from pre-separation column.

**This group factors in fraction 1 [which is not ordinarily analyzed] and material not recovered from pre-separation column.

As an example of the type of analytical data that is resolved from this type of sample, Table 28 details the information obtained from residual oil 1 in terms of apparent molecular weight of a species and a semi-quantitative estimate of its occurrence in the as-received sample. Among the 340 entries there would be a certain, probably relatively minor, degree of duplication representing GC peak overlaps. Quite a few of the GC peaks as enhanced by computer appear to be dominated by single components; some are multi-component as shown by the spacing used in the table. In the column headed "apparent Molecular Weight", listings in parentheses show mass peaks that may be associated with a "molecular weight". In general these denote an apparent loss of a methyl group from the molecular ion. (Due to many factors, the lack of table entry at parent ion minus 15 should not be given undue importance.) In one case (entry 226) this mass association, if valid, indicates a loss of a hydroxyl ion or ammonia. Entries in brackets in this table column may not be molecular weights. They signify occurrence of dominant peaks that are not readily rationalized in terms of other peaks present. They could signify a dominant peak in a mass spectrum with insignificant parent ion or parent ion heavier than 320 mass units (e.g., entry 212 represents dioctylphthalate - M.W. 390). The odd number bracket entries could signify the molecular weight of ions containing an odd number of nitrogen atoms. Or, bracketed entries could simply signify forced operation of computer peak enhancement.

In Table 28, column titled "Level of Occurrence Suggested," listings are normalized to an "as-received sample" basis. They are intended to be only semi-quantitative and are quoted on the basis of 30 ion counts in a GC peak per ppm as affected by concentration factors involved in sample

Table 28

GC/MS ANALYSIS OF RESIDUAL FUEL OIL FRACTIONS

Sample:	Residual Oil #1	Shell #6
	High Sulfur Fuel Oil	Code 41010

Entry No.	Approx. Retention Time	Apparent Molecular Weight	Occurrence in Fraction No. 2	3	4	5	6	7	Level of Occurrence Suggested	Remarks
1	8.4	142	✓						250 ppm	
2	8.6	142	✓						325	
3	9.7	154	✓						285	
4	10.0	156 (141)	✓						480	
5	10.1	156 (141)	✓	✓					2,200	3.5 ppm in Fraction 3
6	10.3	156 (141)	✓	✓					5,800	6 ppm in Fraction 3
7	10.3	162 (147)				✓			0.7	
8	10.6	162(147)				✓			} 1	
9		206				✓				
10	10.7	156 (141)	✓						810	
11	10.9	156 (141)	✓						820	
12	11.4	168 (153)	✓	✓					1,750	4.5 ppm in Fraction 3
13	11.7	170 (155)	✓						6,000	
14	11.9	220 (205)			✓				15	
15	11.9	176 (161)				✓			0.3	
16	12.0	170 (155)	✓	✓					2,100	36 ppm in Fraction 3
17	12.1	170(155)	✓						3,000 ppm	
18	12.4	170(155)	✓						170	
19	12.4	154		✓					10	
20	12.5	176				✓			0.6	
21	12.6	170(155)	✓						1,750	
22	13.0	166	✓						} 2,300	
23		182	✓							
24	13.1	160				✓			} 0.5	
25		166				✓				
26		176				✓				
27		190(175)				✓				
28	13.2	168(153)	✓	✓					} 6,300	65 ppm in Fraction 3
29		180	✓							
30		184	✓	✓						
31	13.2	[165]		✓					2	
32	13.4	[149]						✓	0.003	
33	13.5	184(169)	✓						120	
34	13.6	182	✓						500	
35	13.9	182	✓						} 95	
36		184(169)	✓							

Table 28 (Cont)

Entry No.	Approx. Retention Time	Apparent Molecular Weight	Occurrence in Fraction No.						Level of Occurrence Suggested	Remarks
			2	3	4	5	6	7		
37	14.3	184(169)	✓	✓					1,550 ppm	9 ppm in Fraction 3
38		210	✓	✓						
39		196		✓						
40	14.5	184(169)	✓						2,000	
41		196	✓							
42	14.7	180(165)	✓	✓		✓			1,500	35 ppm in 3; 0.6 in 5
43		204(189)				✓				
44	14.8	182(167)	✓						3,250	
45		194(179)	✓							
46		198	✓							
47	15.5	120(105)	✓						65	
48		184	✓							
49		224	✓							
50	15.8	178	✓	✓		✓			1,200	1 ppm in 3; 8 ppm in 6
51	16.4	194(179)	✓	✓					165	1 ppm in 3
52		210	✓	✓						
53	16.6	106(91)	✓						185	
54		120	✓							
55		222	✓							
56		238	✓							
57	16.8	198	✓			✓	✓		880	17 ppm in 5; 3 in 6
58		210	✓							
59	17.1	198				✓	✓		65	2 ppm in Fraction 6
60	17.4	192	✓	✓	✓	✓	✓	✓	5,550	5,545 ppm in Fraction 2
61	17.7	192	✓						4,400 ppm	
62	17.9	208(193)		✓					10	
63	18.1	[149]						✓	0.1	
64	18.1	212	✓						110	
65	18.0	198				✓			0.3	
66		212(197)				✓				
67	18.3	212(197)	✓			✓	✓		70	10 ppm in 2; 4 in 6
68	18.3	212	✓						55	
69	18.5	208(193)		✓					185	
70	18.6	212				✓			130	
71	18.7	212(197)					✓		10	
72	18.8	206(191)	✓	✓		✓			5,700	16 ppm in 3; 0.5 in 5
73	18.9	206			✓				2	
74	19.1	206(191)	✓	✓	✓	✓	✓		10,500	14 ppm in 3; 0.5 in 4; 5 in 5; 1 in 6
75		222(207)		✓						
76		226			✓					
77	19.4	226(211)				✓			1	
78	19.5	206	✓						110	
79	19.7	220(205)	✓						250	0.3 ppm in 5; 40 in 6
80		226(211)	✓			✓	✓			

Table 28 (Cont)

Entry No.	Approx. Retention Time	Apparent Molecular Weight	Occurrence in Fraction No.						Level of Occurrence Suggested	Remarks
			2	3	4	5	6	7		
81	19.8	222(207)		✓					95	
82	19.8	220(205)	✓			✓			50	20 ppm in Fraction 5
83		226(211)				✓				
84	20.1	202	✓						2,250	33 ppm in 3; 1 in 5
85		220(205)	✓	✓		✓				
86		226(211)				✓				
87	20.1	210				✓			2	
88	20.2	212(197)				✓			1.5	0.5 ppm in Fraction 5
89		226				✓	✓			
90		240				✓				
91		280				✓				
92	20.4	220(205)	✓	✓	✓	✓	✓	✓	5,850	5,800 ppm in Fraction 2
93		226(211)					✓			
94		240			✓	✓				
95	20.5	240(225)				✓	✓		15	1 ppm in Fraction 6
96	20.7	240(225)				✓			10	
97	20.8	224				✓			1	
98	20.9	[102]	✓						15	
99		[120]	✓							
100		220	✓							
101		232	✓							
102	21.0	240(225)					✓	✓	10	9^+ ppm in Fraction 5
103		aliphatic				✓	✓	✓		
104	21.0	220(205)	✓						55 ppm	
105		[225]	✓							
106		234	✓							
107	21.0	194		✓					75	
108		208		✓						
109		236(221)		✓						
110	21.1	216	✓						40	
111		234(219)	✓							
112	21.2	234(219)	✓						20	
113	21.3	240(225)				✓			3	
114	21.4	240(225)				✓			1	
115		254				✓				
116		268				✓				
117	21.4	226				✓			0.5	
118	21.4	202		✓					5	
119		218(203)		✓						
120		236(221)		✓						
121	21.5	216	✓			✓			1,750	6 ppm in Fraction 5
122		222				✓				
123		240(225)				✓				
124		254(239)				✓				
125	21.5	[221]		✓					30	

Table 28 (Cont)

Entry No.	Approx. Retention Time	Apparent Molecular Weight	Occurrence in Fraction No.						Level of Occurrence Suggested	Remarks
			2	3	4	5	6	7		
126	21.6	220				✓			10 ppm	1 ppm in Fraction 5
127		240(225)				✓	✓			
128		254(239)				✓	✓			
129	21.7	216	✓				✓	✓	2,600	2 ppm in 6; 0.2 in 7
130		240(225)					✓	✓		
131		254(239)						✓		
132	21.7	224			✓				1	
133	21.8	234(219)		✓					25	
134	21.8	[225]				✓			2	
135		254(239)				✓				
136	22.0	[149]						✓	0.02	
137	22.1	234(219)	✓	✓					730	20 ppm in Fraction 2
138		248	✓							
139	22.1	224					✓		70	30 ppm in Fraction 5
140		240(225)					✓			
141		254(239)					✓			
142		aliphatic				✓	✓			
143	22.4	236(221)				✓			0.3	
144	22.4	230(215)	✓	✓		✓			35	5 ppm in 2; 1.5 in 5
145		248	✓	✓						
146		268	✓							
147		254(239)				✓				
148		268(253)				✓				
149	22.5	202	✓						125 ppm	8 ppm in Fraction 3
150		230(215)	✓	✓						
151		232(217)	✓							
152	22.6	232(217)	✓						20	
153	22.7	230(215)	✓	✓			✓		120	60 ppm in 3; 0.5 in 6
154		232	✓	✓						
155		236(221)					✓			
156		246	✓	✓						
157		248		✓						
158	22.7	254(239)					✓		4	
159	22.8	230	✓						30	
160		232(217)	✓							
161		[235]	✓							
162	22.8	230	✓						45	
163	22.9	216					✓		1	
164		220					✓			
165	23.0	230(215)	✓						40	
166		246	✓							
167	23.1	230	✓			✓		✓	560	11 ppm in 5; 203 in 6; 0.2 in 7
168		234	✓			✓				
169		268				✓				
170		aliphatic				✓	✓	✓		

Table 28 (Cont)

Entry No.	Approx. Retention Time	Apparent Molecular Weight	Occurrence in Fraction No. 2	3	4	5	6	7	Level of Occurrence Suggested	Remarks
171	23.1	[107.6]		✓					35 ppm	
172	23.3	230(215)	✓						105	3.5 ppm in Fraction 6
173		240					✓			
174		250	✓				✓			
175		268					✓			
176	23.3	248(233)		✓					7	
177	23.4	254(239)					✓	✓	1.5	0.1 ppm in Fraction 7
178	23.5	[215]		✓					15	
179	23.6	[249]					✓		0.5	
180	23.7	220					✓		0.5	
181		252(237)					✓			
182		258(243)					✓			
183		266					✓			
184		286(271)					✓			
185	23.7	208					✓		1	
186	23.7	228	✓						30	
187		[235]	✓	✓						
188		244(229)	✓	✓	✓					
189		246	✓		✓					
190		260	✓							
191		276	✓							
192	23.8	228	✓						3,400	62 ppm in Fraction 2
193		244	✓	✓						
194		260		✓						
195	23 8	228	✓			✓			25 ppm	2 ppm in Fraction 5
196		250				✓				
197		266(251)				✓				
198		268(253)				✓				
199	23.9	250(235)				✓			0.5	
200		266				✓				
201		268				✓				
202		282				✓				
203	24.0	202				✓			1 ppm	
204		228				✓				
205		[239]				✓				
206	24.0	236				✓			0.7	
207		250				✓				
208	24.2	248				✓			4	
209		250(235)					✓			
210		266					✓			
211		aliphatic				✓	✓	✓		
212	24.4	[149]						✓	25	
213	24.1	[227]	✓						20	
214	24.6	[229]	✓	✓					75	
215	24.9	264(249)					✓	✓	1	
216		272					✓			
217	25.0	242	✓	✓		✓			140	17 ppm in 2; 6 in 5
218		258		✓						

Table 28 (Cont)

Entry No.	Approx. Retention Time	Apparent Molecular Weight	Occurrence in Fraction No. 2	3	4	5	6	7	Level of Occurrence Suggested	Remarks
219	25.2	242(227)	✓	✓		✓	✓	✓	175 ppm	70 in 2; 1.5 in 5; 3 in 6; 0.02 in 7
220		258		✓			✓	✓		
221		262				✓				
222		aliphatic				✓	✓	✓		
223	25.2	242	✓						15	
224	25.5	262(247)				✓	✓		1	
225	25.6	[229]		✓					85	
226	25.7	[241]		✓					15	
227	25.7	262(245)				✓			3	
228	25.8	244					✓		3	
229		262					✓			
230		282					✓			
231		302					✓			
232		308					✓			
233	25.8	262					✓		0.7	
234		268					✓			
235		280					✓			
236		284					✓			
237		296					✓			
238	26.0	[261]				✓			1	
239	26.1	244			✓				0.4	
240		258			✓					
241		264			✓					
242	26.1	262				✓			0.5	
243	26.2	262				✓			1.5 ppm	
244		290				✓				
245	26.2	268				✓			0.7	
246	26.3	256(241)		✓					8	
247	26.5	256(241)		✓					85	
248	26.5	220				✓			1.5	
249		260(245)				✓				
250		262				✓				
251	26.5	256(241)		✓					8	
252	26.6	256(241)		✓					7	
253		258		✓						
254	26.6	256(241)		✓					10	
255	26.7	[229]		✓					20	
256		256		✓						
257	26.8	242		✓					10	
258		258		✓						
259		268(253)		✓						
260	26.9	256(241)	✓			✓			60	2 ppm in Fraction 5
261		262				✓				
262	27.0	[257]		✓					70	
263	27.1	256				✓			1	
264		276(261)				✓				
265	27.3	[261]				✓	✓		5	
266	27.5	[215]		✓					4	

Table 28 (Cont)

Entry No.	Approx. Retention Time	Apparent Molecular Weight	Occurrence in Fraction No. 2	3	4	5	6	7	Level of Occurrence Suggested	Remarks
267	27.6	276(261)					✓		0.3 ppm	
268	27.8	270(255)		✓					20	
269	27.9	[229]				✓			0.5	
270	28.0	270		✓					15	
271		276		✓						
272	28.2	270		✓					35	
273	28.4	270		✓					10	
274	28.5	[127]		✓					25	
275	28.5	[255]		✓					25	
276		272		✓						
277	28.7	264		✓					20	
278		270(255)		✓						
279	28.9	270(255)		✓					10	
280	29.1	[127]		✓					20	
281	29.1	256		✓					4	
282		270		✓						
283	29.2	[134]		✓					10	
284		202		✓						
285		270		✓						
286	29.3	284		✓					15	
287	29.5	270		✓					6	
288	29.7	290					✓		4	
289	29.7	282					✓		1 ppm	
290		304					✓			
291	29.7	284(269)		✓					10	
292	29.9	[127]		✓					15	
293	30.0	290(275)		✓					20	
294		300		✓						
295	30.0	[253]		✓					8	
296	30.1	[128]		✓					10	
297		[130]		✓						
298	30.1	226		✓					7	
299		238		✓						
300		256		✓						
301	30.2	258		✓					1	
302		290		✓						
303	30.3	[128]		✓					15	
304	30.4	[127]		✓					10	
305	30.7	284		✓					4	
306	30.9	[120]		✓					7	
307	31.2	[132]		✓					10	
308	31.4	[127]		✓					25	
309	31.5	284		✓					30	

Table 28 (Cont)

Entry No.	Approx. Retention Time	Apparent Molecular Weight	Occurrence in Fraction No. 2	3	4	5	6	7	Level of Occurrence Suggested	Remarks
310	31.6	[127]		✓					7 ppm	
311	31.7	226		✓					10	
312		260		✓						
313	32.0	266		✓					10	
314		[269]		✓						
315	32.1	[134]		✓					10	
316	32.2	252		✓					10	
317		262		✓						
318		266		✓						
319		282		✓						
320		290		✓						
321		298		✓						
322	32.6	[133]		✓					10	
323	32.9	[130.4]		✓					15	
324	33.0	[119.4]		✓					15	
325	33.2	[255]		✓					10	
326		[279]		✓						
327	33.5	256		✓					7	
328	34.1	280		✓					15	
329	34.3	[131]		✓					5	
330	35.7	280(265)		✓					10	
331	35.8	[269]		✓					10	
332		280		✓						
333	36.2	280		✓					0.7 ppm	
334	37.3	280(265)		✓					7	
335	38.0	[133]		✓					7	
336	38.3	280		✓					4	
337		302		✓						
338	39.6	[133]		✓					10	
339	39.9	282					✓		1.5	
340		300					✓			

pre-treatment. For the fractions from oil 1, the "1 ppm" thresholds are as follows:

Fraction 1-2	150	ion counts per ppm
1-3	150	"
1-4	250	"
1-5	600	"
1-6	600	"
1-7	6,000	"

Another point is illustrated by the data of the table. A particular sample component can be found in more than one pre-separation fraction. Usually it is of greatest abundance in the earliest fraction. Other evidence (as in Appendix F) shows that a component can be clearly isolated in a particular fraction from one oil and just as clearly into a different fraction from a different oil. Sometime a complete pre-separation fraction of one oil run gives a GC pattern similar to a different fraction from a different oil [see GC traces of oil 11, fraction 4 and oil 12, fraction 5; figures F-7 and F-8 respectively . The only explanation for this phenomenon that we offer at present is that, at a 4 gram column load, the nature of the sample being eluted affects the elution process.

Another phenomenon is the way the same sample components can be found in several column chromatography fractions of the same oil. Because of the GC peak pattern they produce, this is most noticeable with aliphatic hydrocarbons. Westinghouse Oil Sample 16 was studied to determine if there was any pattern to this. The study results (Table 29) show that there is no one major pattern in this behavior, although it should be pointed out that on an "as-received" sample basis, the effect appears to be stronger with higher molecular weight material, most of the aliphatic hydrocarbons elute into column chromatography Fraction 1. The smooth curve of the data under "Interval Between Retention Times" would seem to indicate a homologous series of aliphatics is involved.

TABLE 29

OCCURRENCE OF ALIPHATICS IN FRACTIONS FROM OIL #16

Retention Time of Aliphatic (minutes)	Interval Between Retention Times (minutes)	"As Injected" Sample Basis					"As-Received" Sample Basis				
		Fraction 1	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 1	Fraction 4	Fraction 5	Fraction 6	Fraction 7
7.9	1.8	150 "ppm"	--	--	--	--	90 "ppm"	--	--	--	--
9.7	1.7	500	--	--	--	--	305	--	--	--	--
11.4	1.5	1100	--	--	--	--	670	--	--	--	--
12.9	1.5	2200	--	--	--	--	1340	--	--	--	--
14.4	1.3	600	10 "ppm"	600 "ppm"	--	--	365	0.2 "ppm"	15 "ppm"	--	--
15.7	1.3	3300	140	800	60 "ppm"	5 "ppm"	2015	3.4	20	0.4 "ppm"	0.01 "ppm"
17.0	1.2	2500	4	1600	60	15	1525	0.1	40	0.2	0.02
18.2	1.2	65	--	1300	300	1300	40	--	30	2	2
19.4	1.2	3	20	1500	650	650	1.8	0.5	35	4	1
20.6	1.1	1100	15	1800	1000	900	670	0.4	45	6	1.5
21.7	1.0	1100	135	1600	1000	150	670	3.2	40	6	0.2
22.7	1.0	1000	40	1200	1050	400	610	1	30	7	0.6
23.7	1.0	1100	27	200	275	--	670	0.6	5	2	--
24.7	1.1	35	--	1200	1300	10	20	--	30	10	0.02
25.8	1.2	--	--	60	20	30	--	--	1	0.1	0.05
27.0	1.7	--	--	35	150	--	--	--	1	1	--
28.7	2.1	--	--	15	140	--	--	--	0.4	1	--
30.8		--	--	--	20	--	--	--		0.1	--

Table 30 is the result of the attempt to identify individual components by computer matching against the computer library of over 35,000 compounds. There were 67 peak entries. Fifty of them are of materials resolved enough to be considered identifiable. Of the 50 computer generated matches, 29 are considered unacceptable based, usually, on the polarity of the "match". The problem would seem to have two sides:

1. The reference data base is too small.
2. The computer matching program is not strong enough to handle variables encountered.

It was felt that the best way to identify components in an oil sample was through visual comparison of sample and reference spectra. The result from such an effort is shown in Table 31.

In Tables 30 and 31 an entry "W" under "Assignment" indicates an oil component whose spectra were weak through contamination with other components or through being forced. It is not very likely that any identification could be performed through such spectra. In general, "weak" spectra are of components of low occurrence. The entry "S" in this column of Table 31 denotes mass spectra of oil components wherein there are strong, higher even mass peaks that suggest identification is possible. It is disappointing that there are so many "S" entries rather than tentative identifications in Table 31. The result of this effort would seem to indicate the visual search is no worse than the computer search and may even be somewhat more effective. The magnitude of the problem is illustrated in Figure 69, where the tragedy is not the poor match the computer is trying to make, but rather that a strong, clean distinctive mass spectrum for the unknown is not represented in the library. [In Figure 69, the reference spectrum is on top; the unknown on the bottom. The upper data in the middle figure is information in the unknown which is not in the reference; below, data in the reference not in the unknown. All spectra are individually normalized.] The material represented by the unknown in Figure 69 (m.w. 216) does not

Table 30

TENTATIVE IDENTIFICATION OF COMPONENTS IN OIL #23, FRACTION 2 (BY COMPUTER LIBRARY SEARCH)

GC Peak Number	Assignment*
1	W
2	1-methylnaphthalene
3	W
4	1,1-dimethyl-1,3,5-trisilacyclohexane
5	2-ethylnaphthalene
6	1,5-; 1-7; 1,3-; 1,6-; or 2,7-dimethylnaphthalene
7	1,8-dimethylnaphthalene
8	(1-methyl-N-nitro-2(1H)-pyridimine)
9	(Biphenylene)
10	2-(1-methylethyl)-naphthalene
11	1,6,7- or 2,3,6-trimethylnaphthalene
12	1,6,7- or 2,3,6-trimethylnaphthalene
13	1,6,7- or 2,3,6-trimethylnaphthalene
14	W
15	1 H phenalene and 3-methyl-1,1'-biphenyl
16	(hexahydrofuropyrolizinone)
17	5-ethyl-1,3-dimethyl-5-(1-methylpropyl)-2,4,6-pyrimidiretrione
18	(4-amino-N-2-pyridinyl-benzenesulfonamide)
19	([1,1'-biphenyl]-4,4'-diamine)
20	([1,1'-biphenyl]-2-ol)
21	(5,7-dihydro-6H-dibenzo[a,c]cyclohepten-6-one)
22	(9 H-fluoren-9-one)
23	([1,1'-biphenyl]-3-acetic acid)
24	(dibenzothiophene, 5-oxide)
25	phenanthrene
26	(bis(3,5-dimethylbenzyl)sulfide)
27	(2,2-diethyl-4,4-diphenyl-1,3-oxathiolan-5-one)
28	(N,N'-diethyl-1,3,5-triazine-2,4,6-triamine)
29	(3,3',4,4'-tetramethyl-1,1'-biphenyl)

---

*An assignment in parentheses indicates an unacceptable match.

Table 30 (Cont)

GC Peak Number	Assignment
30	(3-(4-biphenylyl)-1-phenyl-2-pyrazoline)
31	(8-(methylthio)-1H-pyrine)
32	3-methyldibenzothiophene
33	4-methyldibenzothiophene
34	4-methylphenanthrene
35	4-methylphenanthrene
36	(2-(methylseleno)-5-(methylthio)-thiophene
37	(·alpha·-methylenebenzeneacetonitrile)
38	(1,2-dihydro-4-phenylnaphthalene)
39	2,5-dimethylphenanthrene
40	2,7 or 3,6-dimethylphenanthrene
41	(3,5-bis(1,1-dimethylethyl)-4-hydroxy-2,4-cyclohexadien-1-one
42	2,7-dimethylphenanthrene
43	(20-methyl-5·alpha·-pregnan-15-one)
44	(4-(2-phenylethenyl)-benzonitrile)
45	W
46	pyrene
47	W
48	W
49	1-methylpyrene
50	W
51	(trimethyl-2-naphthalenylsilane)
52	W
53	((cyanoquanidinato-N')methylmercury)
54	(4-methyl-2,5-diphenyl-1,3,2-oxazaborolidine)
55	W
56	W
57	W
58	(tributylethenylstannane)
59	(4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-methylethenyl)-2-naphthalenone)

Table 30 (Cont)

GC Peak Number	Assignment
60	W
61	(2-(4H-borino[3,2-b:6,7-6']dithien-4-yloxy)-ethanamine)
62	W
63	W
64	W
65	(4,5-epoxy-17-methyl-morphinan-3,14-diol)
66	W
67	W

Table 31

TENTATIVE IDENTIFICATION OF COMPONENTS IN OIL #1, FRACTION 2

[By Visual Comparison]

GC Peak Number	Assignment
1	methylnaphthalene
2	methylnaphthalene
3	biphenyl
4	ethylnaphthalene
5	dimethylnaphthalene
6	dimethylnaphthalene
7	dimethylnaphthalene
8	dimethylnaphthalene
9	ethylnaphthalene
10	3-methylbiphenyl
11	methylethylnaphthalene
12	trimethylnaphthalene
13	methylethylnaphthalene
14	trimethylnaphthalene
15	trimethyl or methylethylnaphthalene
16	phenalene and dimethylbiphenyl
17	6-methyl-6-phenylfulvene (and M.W.s 180 and 184)
18	1-methyl-7-(1-methylethyl)-naphthalene
19	dimethylbiphenyl (and M.W. 184)
20	9H-xanthene or 4-methyldibenzofuran
21	S
22	S
23	4-methylfluorene
24	S
25	phenanthrene or anthracene

Table 31 (Cont)

GC Peak Number	Assignment
26	2,3-dimethylfluorene (and M.W. 210)
27	W
28	methyldibenzothiophene
29	methylphenanthrene or methylanthracene
30	methylphenanthrene or methylanthracene
31	4,9-dimethylnaphtho[2,3-B]thiophene
32	4,9-dimethylnaphtho[2,3-B]thiophene
33	4,9-dimethylnaphtho[2,3-B]thiophene
34	dimethylphenanthrene
35	2-ethylanthracene or 9,10-dimethylphenanthrene
36	W
37	S
38	S
39	fluoranthene or pyrene
40	2,3,5-trimethylphenanthrene
41	W
42	S
43	W
44	S
45	S
46	S
47	1-methylpyrene (and M.W.s 202 and 218)
48	W
49	2,3-benzofluorene
50	S
51	1,2-diphenylbenzene (and M.W. 248)
52	S
53	S
54	S

Table 31 (Cont)

GC Peak Number	Assignment
55	W
56	S
57	S
58	S
59	W
60	S
61	S
62	naphthacene
63	S
64	W
65	W
66	methylbenzo(A)anthracene or methylchrysene
67	methylbenzo(A)anthracene or methylchrysene
68	methylbenzo(A)anthracene or methylchrysene
69	W

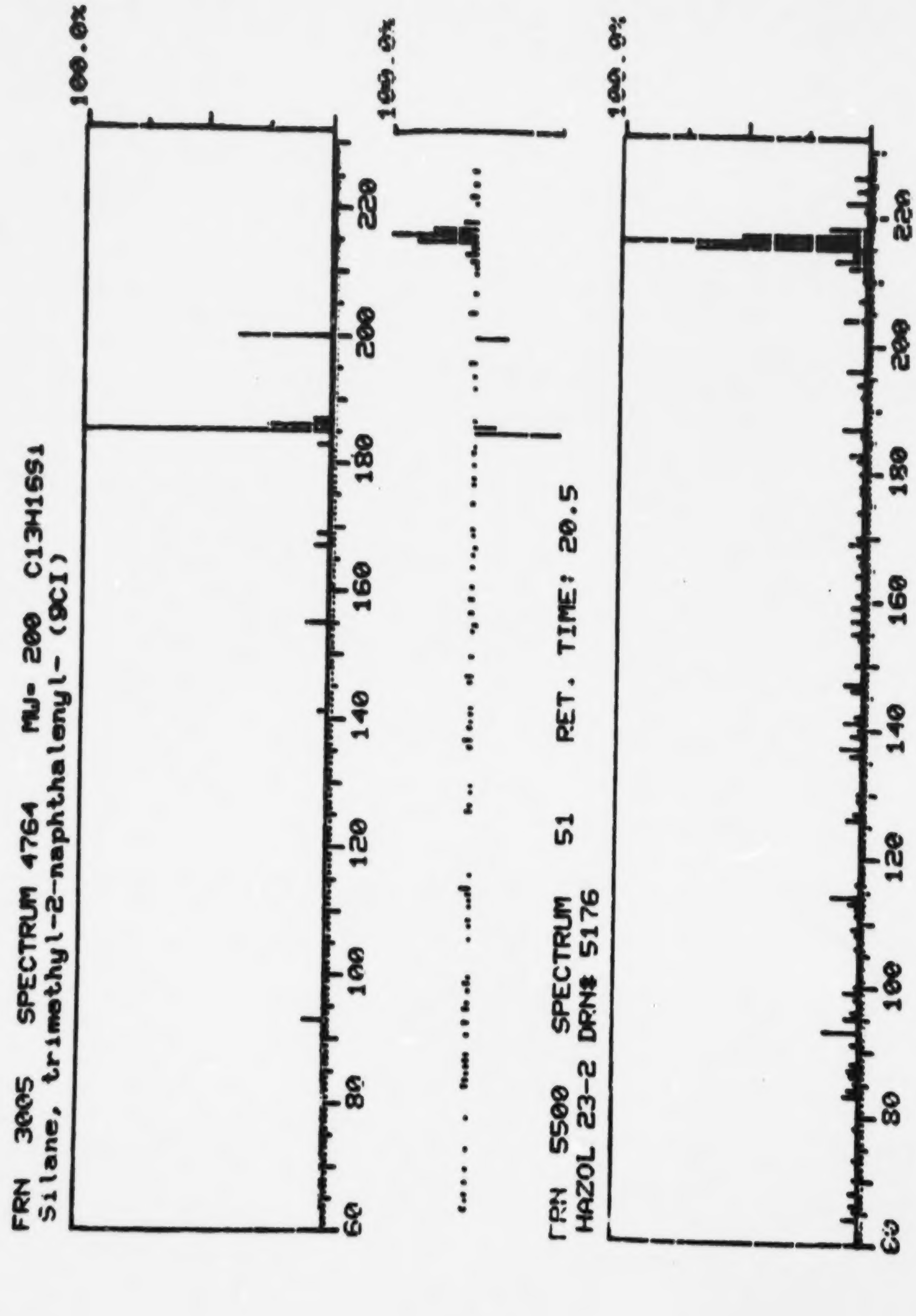


Figure 69. Obvious Mis-matching of Reference and Unknown Spectra

appear to be rare in residual oils. The fact that some oil components isolated are not represented in the library may mean they are fairly unique which would increase the importance of identifying them. There is no way all unmatched spectra obtained in all fractions from an oil could be identified under the intent or life of this contract when there are no legitimate library comparisons.

It was also hoped that when a GC peak represented a mixture, computer subtraction of the mass spectra of the major component could yield a "remainder" with available information. This is shown in Figures 70 and 71. Figure 70 shows the major component of a GC peak "legitimately" identified as phenanthrene. The operation performed for the middle portion of the figure shows substantial information remaining. Using the appropriate software, this information can be reconstructed as a new spectrum and itself searched against the library (Figure 71). Unfortunately, there is once again no library match for what appears to be very real spectra -- of an alkylated organic of molecular weight 194 that loses methyl and ethyl groups in the mass spectrometer. This apparent lack of a data base is most critical with components from pre-separation fractions other than fraction 2.

There is great similarity among oil samples in that certain molecular weights are consistently seen. There are also differences. This can be exemplified by the data of Tables 32 and 33. The body of data from having put 6 pre-fractions each from 23 residual fuel oils through the GC/MS is enormous. In order to make data handling more manageable, only fractions shown to be correlated to mutagenicity should be examined in any detail.

Appendix F contains a more detailed discussion of the GC/MS of pre-separation fractions from several residual oil samples.

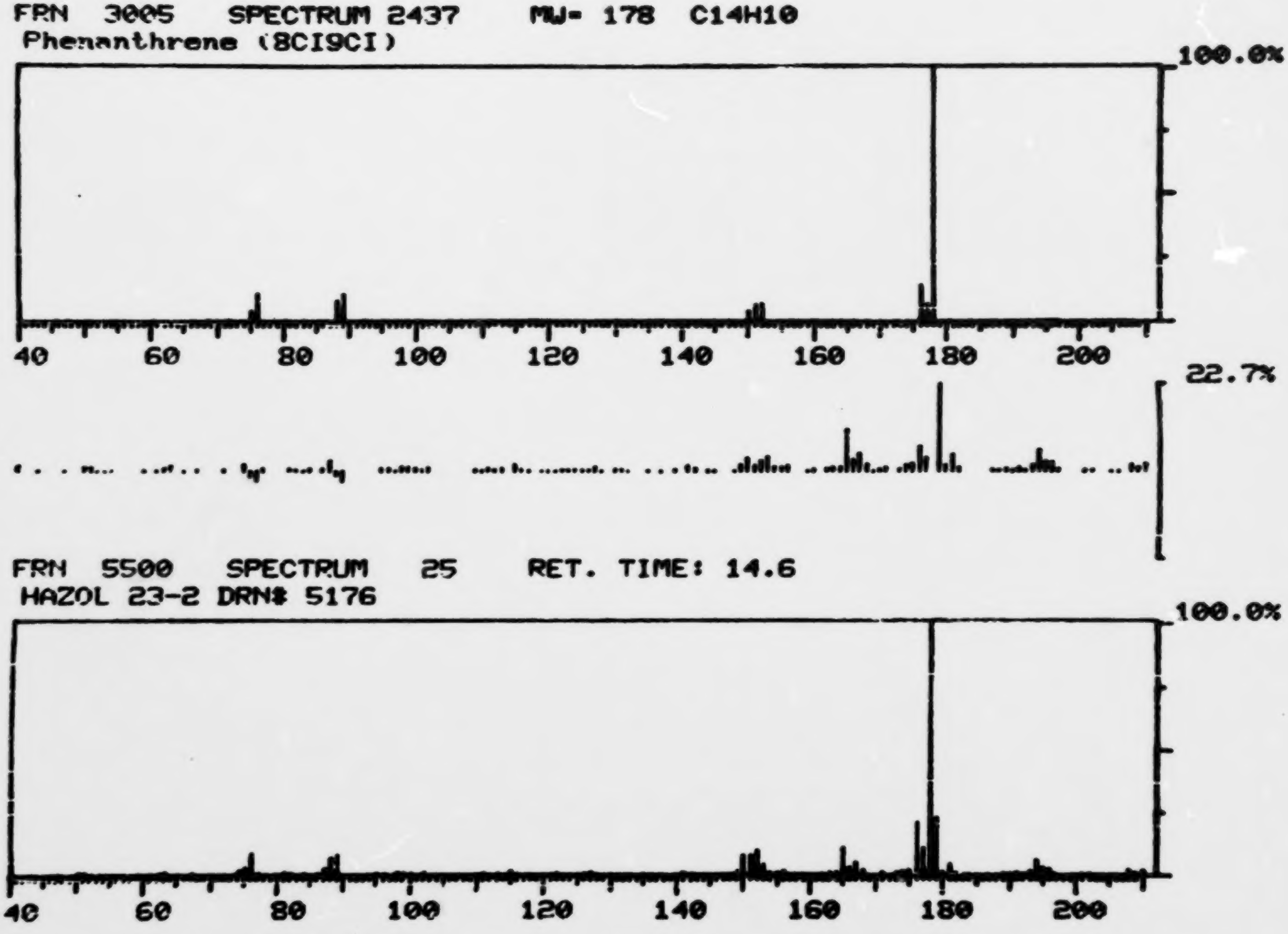


Figure 70. Use of Spectral Subtraction to Show Additional Components in an Unknown

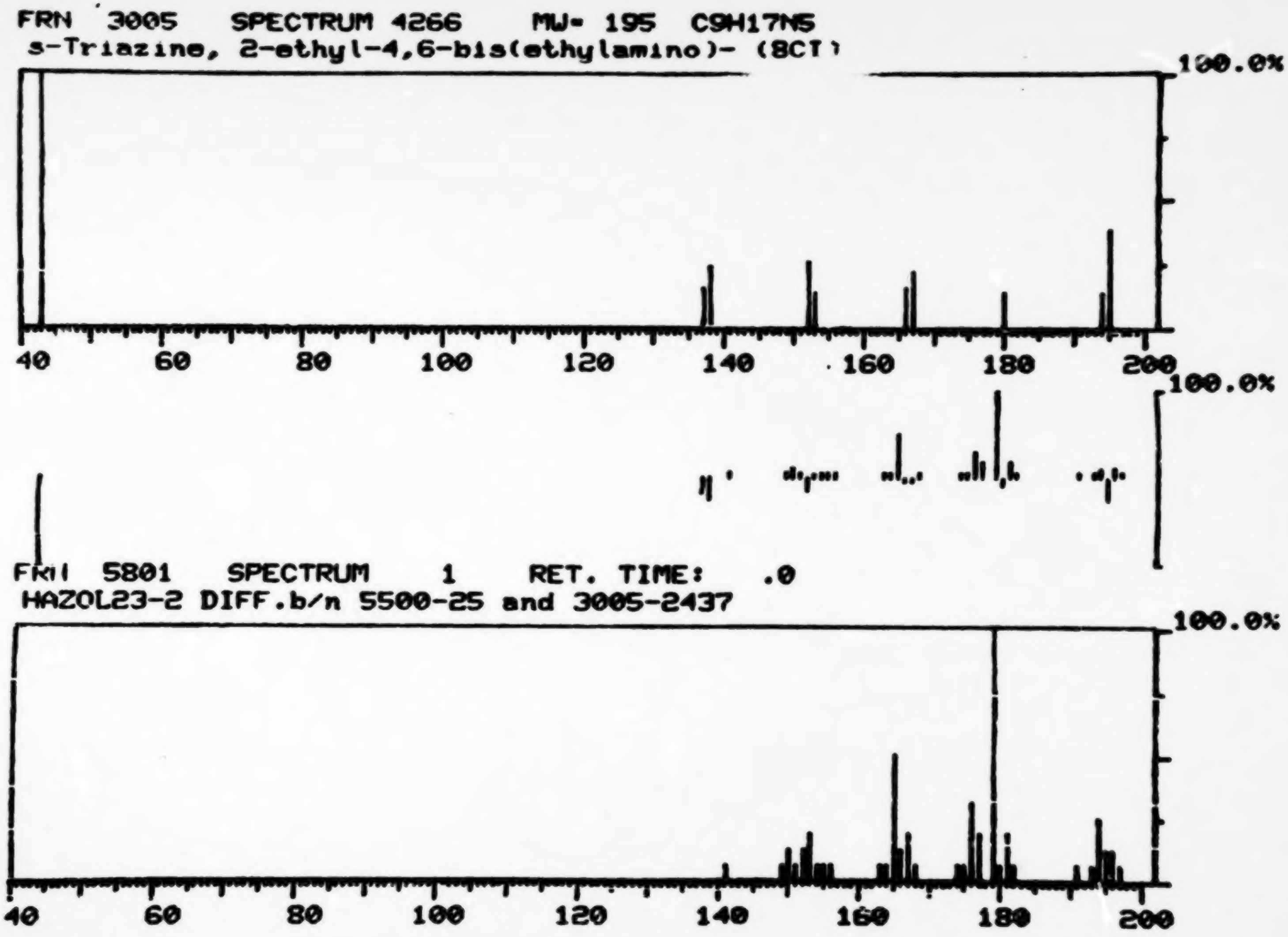


Figure 71. Reconstruct and Search of Spectral "Remainder" Against Computer Library

Table 32

CORRELATION OF COMPONENTS IN RESIDUAL OIL #1 AND #2

Apparent Molecular Weights as Found in:

	Oil #1	Oil #2		Oil #1	Oil #2		Oil #1	Oil #2
1.		146	31.	176		61.		152
2.	142	142	32.	170(155)	170(155)	62.	184(169)	184(169)
3.	142	142	33.	166	166	63.	196	196
4.		160(145)	34.		168	64.		210
5.		[159]	35.		170	65.		152
6.	154		36.	182	182(165)	66.		156
7.	156(141)	156(141)	37.	160		67.	180(165)	180(165)
8.	156(141)	156(141)	38.	166		68.		182(167)
9.	156(141)	156(141)	39.	176		69.		198(183)
10.	162(147)		40.		180	70.	204(189)	204(189)
11.	162(147)		41.		184(169)	71.	182(167)	
12.	206		42.	190(175)	190(175)	72.	194(179)	
13.	156(141)	156(141)	43.		196(181)	73.	198	
14.		174(159)	44.		200	74.	120(105)	
15.	156(141)	156(141)	45.	168(153)		75.	184	184
16.		170(155)	46.	180		76.	224	
17.		152	47.	184		77.	178	178
18.	168(153)	168(153)	48.	[165]		78.		[153]
19.	170(155)	170(155)	49.	[149]		79.	194(179)	194(179)
20.		176(151)	50.	184(169)	184(169)	80.	210(195)	210(195)
21.		182(167)	51.	182		81.	106(91)	
22.	220(205)		52.	182		82.	120	
23.		170(155)	53.	184(169)	184(169)	83.	222	
24.	176(161)	176(161)	54.		198(183)	84.	238	
25.	170(155)	170(155)	55.		204	85.	198	198
26.	170(155)	170(156)	56.	184(169)	184(169)	86.	210	
27.		174(159)	57.	196		87.	198	198
28.	170(155)	170	58.		198(183)	88.		210
29.		172	59.		204(189)	89.		[207]
30.	154		60.	210		90.	192	192

Table 32 (Cont)

	Oil #1	Oil #2		Oil #1	Oil #2		Oil #1	Oil #2
91.	192	192	121.	226(211)		151.	[225]	
92.		[191]	122.	202	202	152.	234	234(219)
93.		[193]	123.	220(205)	220(205)	153.		254
94.	208(193)	208(193)	124.	240(225)	240(225)	154.	194	
95.	198		125.	210		155.	208	
96.	212(197)	212(197)	126.	212(197)		156.	236(221)	
97.	[149]		127.	226	226(211)	157.	216	
98.	212	212	128.		236(221)	158.	234(219)	
99.	212(197)	212(197)	129.	240		159.		216
100.	208(193)		130.	280		160.	234(219)	
101.	212	212(197)	131.	220(205)	220(205)	161.	240(225)	
102.	212(197)	212(197)	132.	226(211)		162.	240(225)	
103.	206(191)	206(191)	133.	240	240(225)	163.	254	
104.		208(193)	134.		[189]	164.	268	
105.		224	135.		220	165.	226	
106.	206		136.	240(225)	240	166.	202	202
107.	206(191)	206(191)	137.		220(205)	167.	218(203)	218(203)
108.	222(207)	222	138.		226(211)	168.		220
109.	226	226(211)	139.		270	169.		234(219)
110.		206	140.		178	170.	236(221)	
111.		226	141.	240(225)	240(225)	171.		240(225)
112.		222	142.		222	172.		280
113.		238	143.	224		173.		202
114.	226(211)	226(211)	144.	[102]		174.	216	216
115.		226(211)	145.	[120]		175.	222	222
116.	206		146.	220	220(205)	176.		232
117.	220(205)		147.	232		177.		234
118.	226(211)	226(211)	148.	240(225)	240(225)	178.	240(225)	240
119.	222(207)		149.		[189]	179.	254(239)	254
120.	220(205)		150.	220(205)		180.	[221]	

Table 32 (Cont)

	Oil #1	Oil #2		Oil #1	Oil #2		Oil #1	Oil #2
181.	220		211.		264	241.	248	
182.	240(225)		212.	224		242.	254(239)	
183.	254(239)		213.	240(225)		243.		260
184.	216	216	214.	254(239)		244.		234
185.	240(225)		215.		230(215)	245.		[155]
186.	254(239)		216.		234	246.	230	
187.	224		217.		244	247.	232(217)	
188.		220	218.		260	248.	[235]	
189.	234(219)	234(219)	219.	236(221)	236(221)	249.	216	
190.		240(225)	220.		190	250.	220	
191.		[159]	221.		236(221)	251.	230(215)	
192.	234(219)		222.		254	252.	246	
193.	[225]		223.	230(215)	230(215)	253.	230	
194.	254(239)		224.	248		254.	234	
195.	[149]		225.	268		255.	268	
196.		[217]	226.		178	256.		[235]
197.		190(175)	227.		230(215)	257.	[107.6]	
198.		210	228.		236	258.		[233]
199.		234	229.	254(239)		259.	248(233)	
200.		238(223)	230.	268(253)		260.	254(239)	
201.		240	231.	202	202	261.	230(215)	
202.		260	232.	230(215)	230(215)	262.	240	
203.		262	233.	232(217)	232(217)	263.	250	
204.		268	234.		234	264.	268	
205.	234(219)	234(219)	235.		248	265.	[215]	
206.	248		236.	232(217)		266.	[249]	
207.		216	237.	230(215)		267.	220	
208.		222	238.	232		268.	252(237)	
209.		234(219)	239.	236(221)		269.	258(243)	
210.		260	240.	246		270.	266	

Table 32 (Cont)

	Oil #1	Oil #2
271.	286(271)	
272.	208	
273.	228	
274.	[235]	
275.	244(229)	
276.	246	
277.	260	
278.	276	
279.		226
280.	228	228(213)
281.	244	244(229)
282.		246
283.	260	
284.	250	
285.		228(213)
286.		244(229)
287.		250(235)
288.	266(251)	
289.	268(253)	
290.	250(235)	
291.	266	
292.	268	
293.	282	
294.		230
295.		248
296.	202	
297.	228	
298.	[239]	
299.	236	
300.	250	
301.	248	
302.	250(235)	
303.	266	
304.		216
305.		226
306.		246
307.		260
308.		[229]
309.		248
310.		258
311.	[227]	
312.	[149]	[149]
313.		244
314.		258
315.		230
316.	[229]	
317.		242
318.	264(249)	264
319.	272	
320.	242	242
321.	258	258(243)
322.		274
323.	242(227)	
324.	258	
325.	262	262
326.		[263]
327.	262(247)	262
328.		248
329.		202
330.	[229]	
331.	[241]	
332.	262(245)	
333.	244	
334.	262	
335.	282	
336.	302	
337.	308	
338.	262	
339.	268	
340.	280	
341.	284	
342.	296	
343.	[261]	
344.	244	
345.	258	258(243)
346.	264	
347.	262	
348.	262	
349.	290	
350.	268	
351.		242
352.	256(241)	256(241)
353.		280(265)
354.		286
355.	256(241)	
356.	220	
357.	260(245)	
358.	262	
359.	256(241)	
360.	256(241)	

Table 32 (Cont)

	Oil #1	Oil #2		Oil #1	Oil #2		Oil #1	Oil #2
361.	258		391.	[134]		421.	260	
362.	256(241)		392.	202		422.	266	
363.	[229]		393.	270		423.	[269]	
364.	256		394.	284		424.	[134]	
365.	242		395.	270		425.	252	
366.	258		396.	290		426.	262	
367.	268(253)		397.		272	427.	266	
368.	256(241)		398.	282		428.	282	
369.	262		399.	304	304	429.	290	
370.	[257]		400.	284(269)		430.	298	
371.	256		401.	[127]		431.	[133]	
372.	276(261)		402.	290(275)	290	432.	[130.4]	
373.	[261]		403.	300		433.	[119.4]	
374.	[215]		404.	[253]		434.	[255]	
375.	276(261)		405.	[128]		435.	[279]	
376.	270(255)		406.	[130]		436.	256	
377.	[229]		407.	226		437.	280	
378.	270		408.	238		438.	[131	
379.	276		409.	256		439.	280(265)	
380.	270		410.	258		440.	[269]	
381.	270		411.	290		441.	280	
382.	[127]		412.	[128]		442.	280	
383.	[255]		413.	[127]		443.	280(265)	
384.	272		414.	284		444.	[133]	
385.	264		415.	[120]		445.	280	
386.	270(255)		416.	[132]		446.	302	
387.	270(255)		417.	[127]		447.	[133]	
388.	[127]		418.	284		448.	282	
389.	256		419.	[127]		449.	300	
390.	270		420.	226				

Table 33

TOTAL POSSIBLE NUMBER OF DIFFERENT COMPONENTS DETECTED IN OILS 1 AND 2

Sample	No. of Possible Components Detected
Oil #1	340
Oil #2	204
Oil #1 and #2	449

"Mutagenically Important" Pre-Separation Fractions

Bioassay work performed disclosed that the major bio-activity resides in Fraction 3 of our 7 pre-separation fractions from an as-received oil. Organic analytical effort was, therefore, directed at determining compositional differences between the active Fraction 3 and the immediately prior, biologically inactive Fraction 2. Oil 16 was chosen for this effort since it yielded the most consistently positive "Fraction 3" tests with all bio-test systems. To help in determining if any of the differences to be found were not of importance, a Fraction 3 of minimum bio-activity (from Oil 12) was carried along in parallel (i.e., if a component is not in Fraction 16-2 but is in Fraction 16-3 and 12-3, it probably is still not environmentally important.)

Even though so little of the total available analytical information is recovered from a gas chromatographic column with these types of samples, some column evaluation work was done in attempting to discover compounds in fraction 16-3 that are not present in fractions 16-2 and 12-3. The chromatograms of Figure 72 show the results obtained with three of the columns studied. They show the best through-put is with the OV101 column and the best resolution is with the OV225 column. (The OV101 column probably has become spent by the hard usage it has received on prior work under this contract.)

Comparisons of sample through-puts, GC columns vs direct insertion probe (DIP) (discussed below) are given in Figures 73 through 75. The data for Fraction 16-2 (Figure 73) show the greatest proportion of sample recovered by GC as compared to DIP (25% with the "best" column). The GC columns in general are seeing the various "molecular weights" in about the same relationship as the DIP analysis with some bias against the mass range 200-224 evident with columns OV225 and OV17/210. This mass range has the most abundant ions by DIP analysis. The data for Fraction 16-3 (Figure 74) shows about 12% of the sample passing

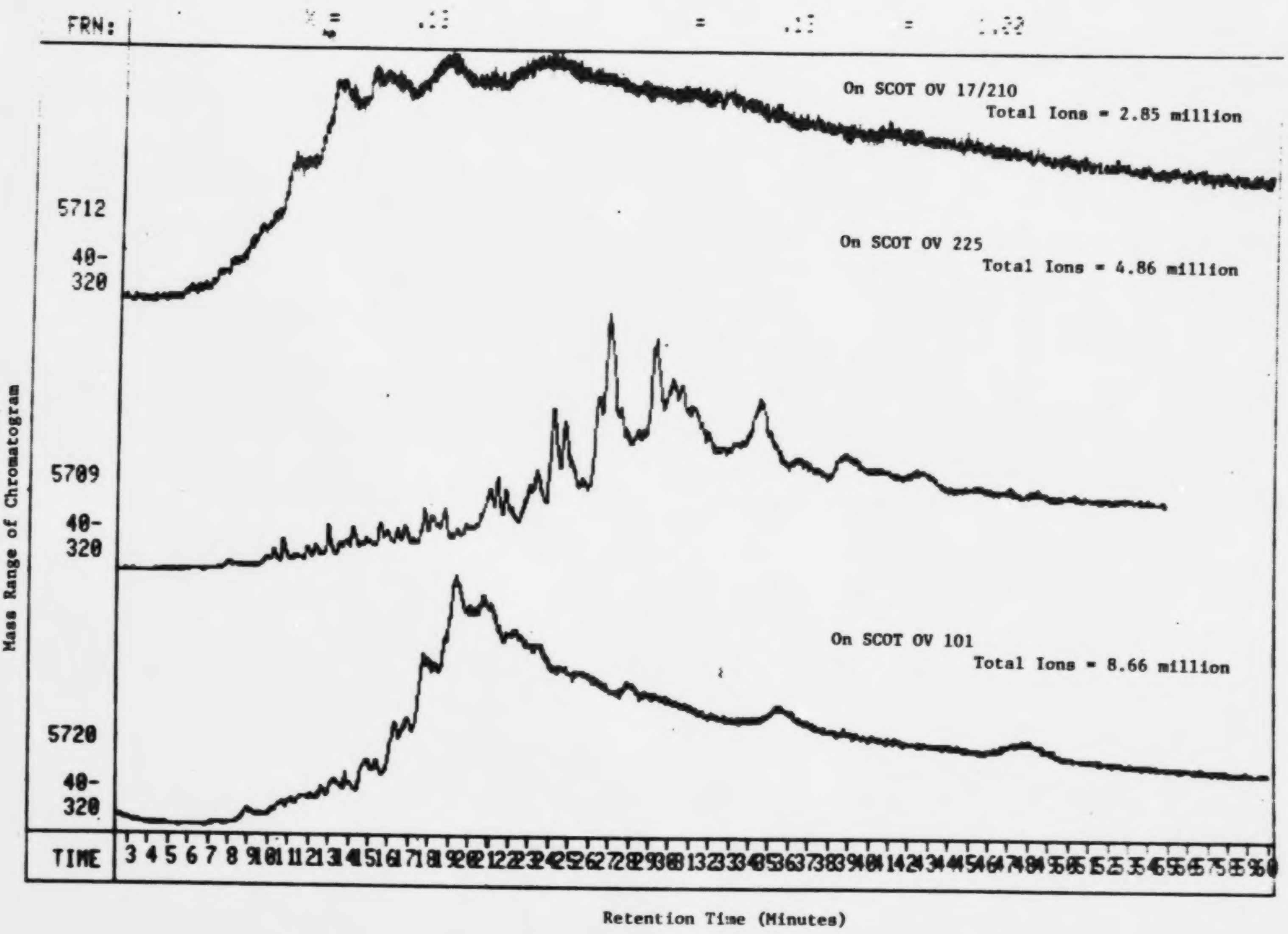


Figure 72. Resolution of Fraction 16-3 on Different Gas Chromatographic Columns

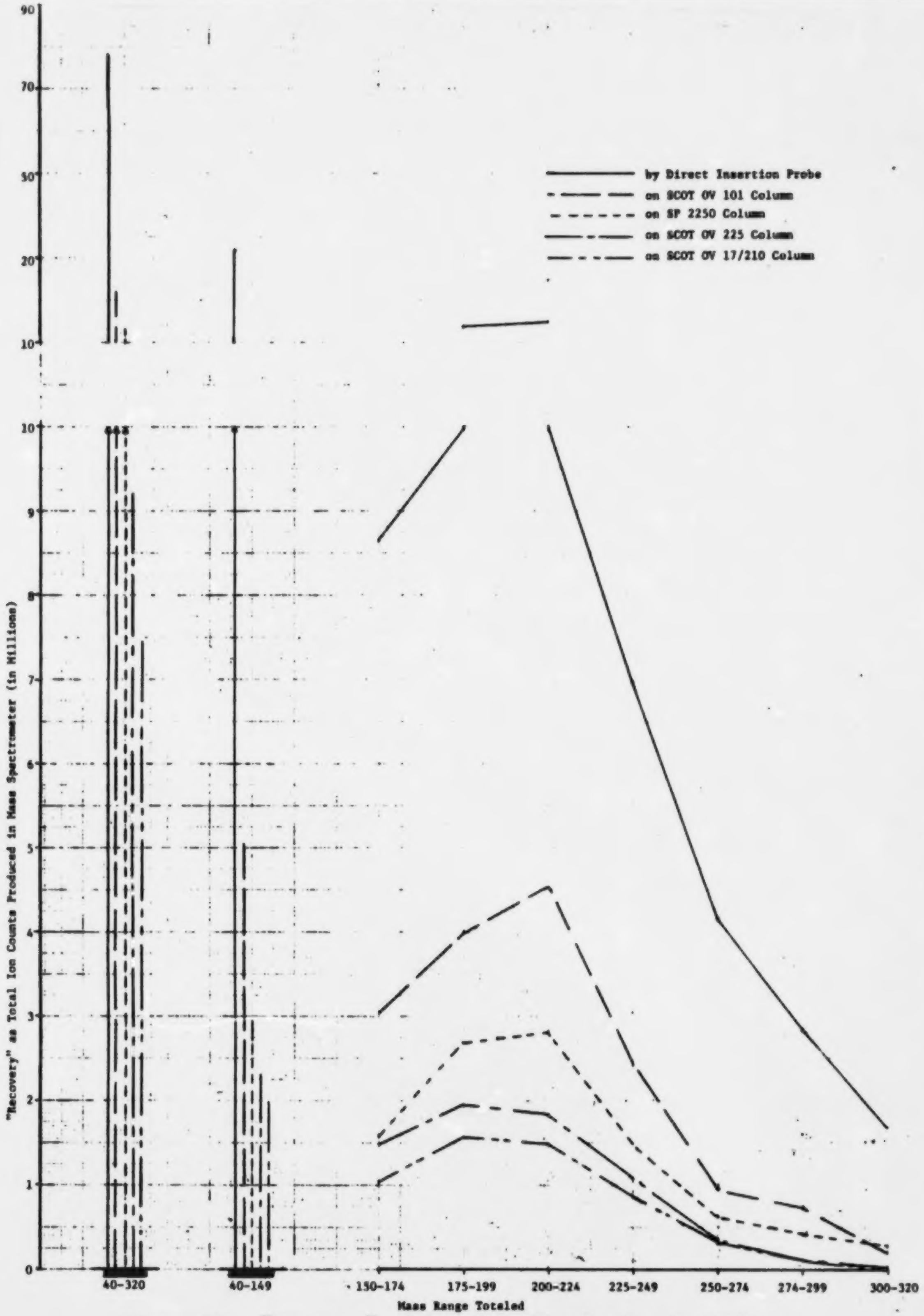


Figure 73. "Recovery" of Fraction 16-2 During Analysis

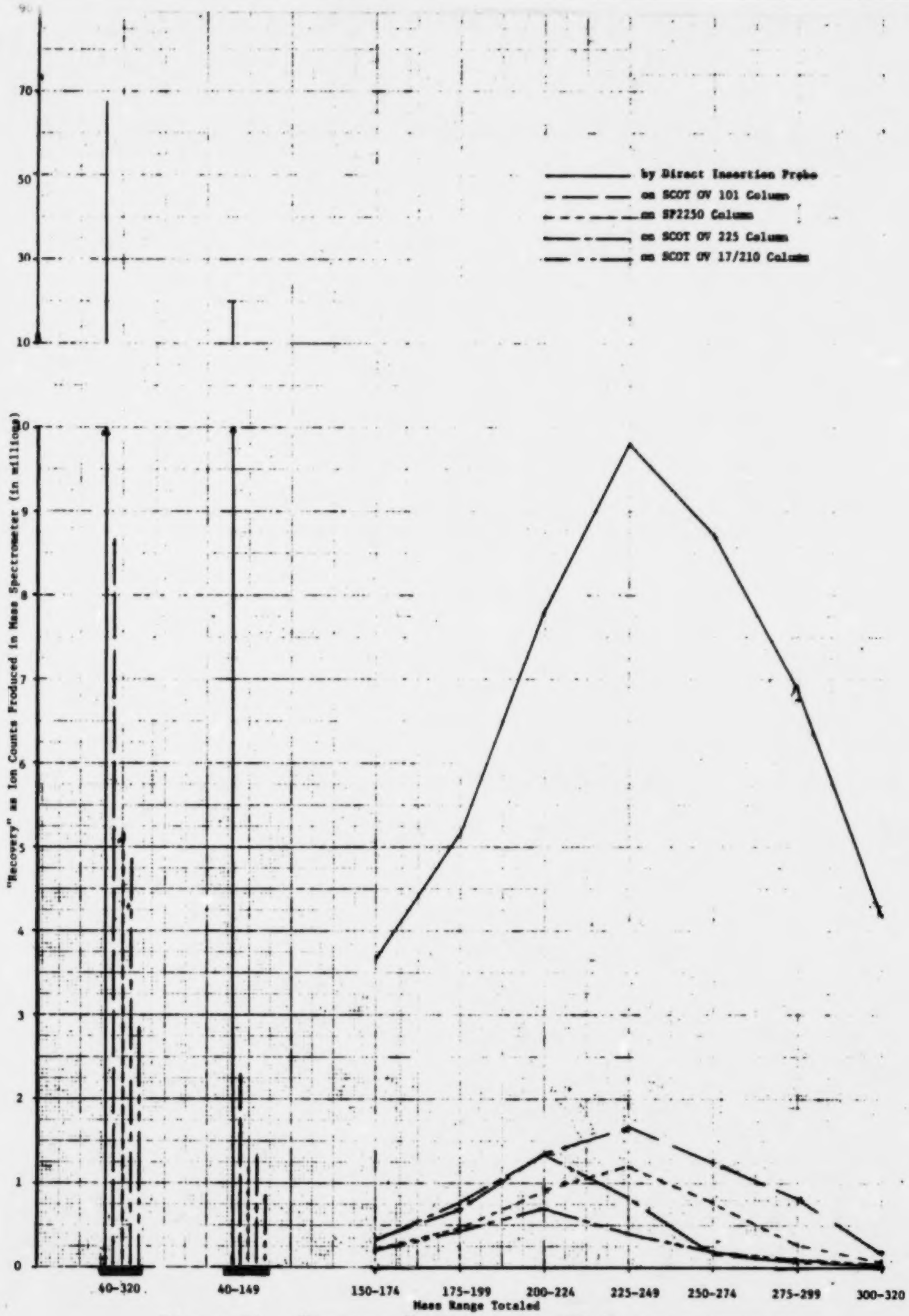


Figure 74. "Recovery" of Fraction 16-3 During Analyses

through the "best" column as compared to the DIP analysis. Columns OV101 and SP2250 appear to be seeing the various "molecular weights" in about the same relationship as the DIP analysis, while columns OV225 and OV17/210 are showing decided bias against material above "molecular weight" of 224. The mass range 225-249 has the most abundant ions according to DIP analysis. The data for fraction 12-3 is given in Figure 75. The "best" column (OV101) is showing about 9% "recovery" compared to DIP analysis. The most abundant ions by DIP are in the 175-199 mass range which is such lower than for the other two fractions. This is as expected since, as mentioned above, Fraction 12-3 is more aliphatic in origin and mass spectra would be more representative of molecular fragments rather than molecular ions. There is no correlation among masses for the various columns used for 12-3 nor against DIP analysis. It is fortunate that Fraction 12-3 has a much lower environmental interest attached. Figure 76 shows the thermogravimetric (TGA) curves for the three fractions to illustrate the major basis for difficulty with GC analysis (i.e., refractory nature of the sample). The recovery variances seen with the different GC columns (Figures 73 through 75) illustrate difficulties based on structure of the components.

There is differential data available in the GC traces of these oil fractions. By plotting restricted mass range chromatograms, it is possible to detect components in Fraction 16-3 that are not in Fraction 16-2. This is shown in Figure 77. Although retention times for the components are different (due primarily to differing helium flow rates for the two runs) it is clear that, qualitatively, the five major GC peaks in the Fraction 16-2 trace are represented in Fraction 16-3. As shown in the inscribed box of Figure 77, 16-3 however has components, whose molecular weights lie in the 175 to 199 range, that are not in Fraction 16-2. As seen in Figure 78, the OV101 column, at least in its present condition, is not showing this resolution.

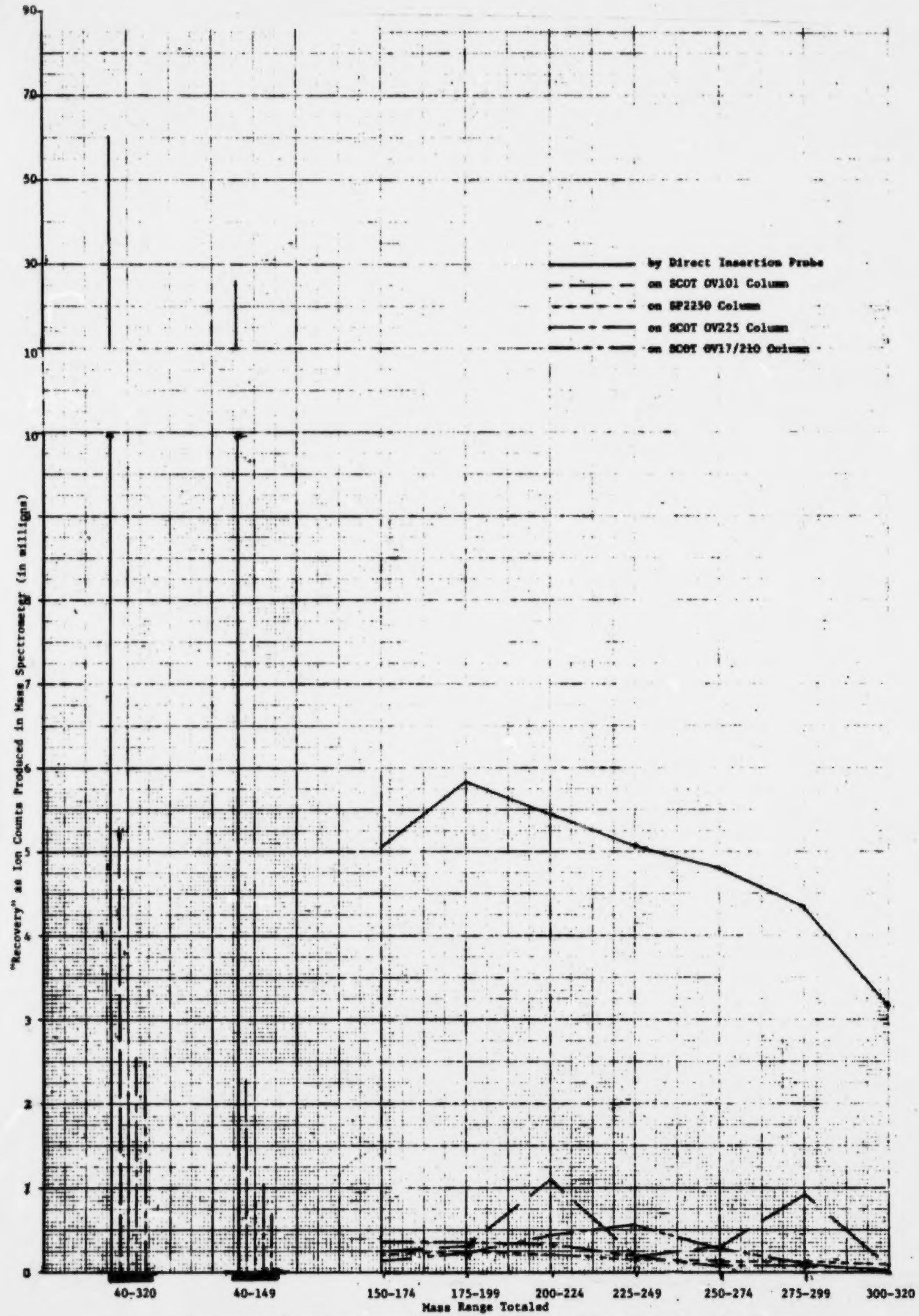
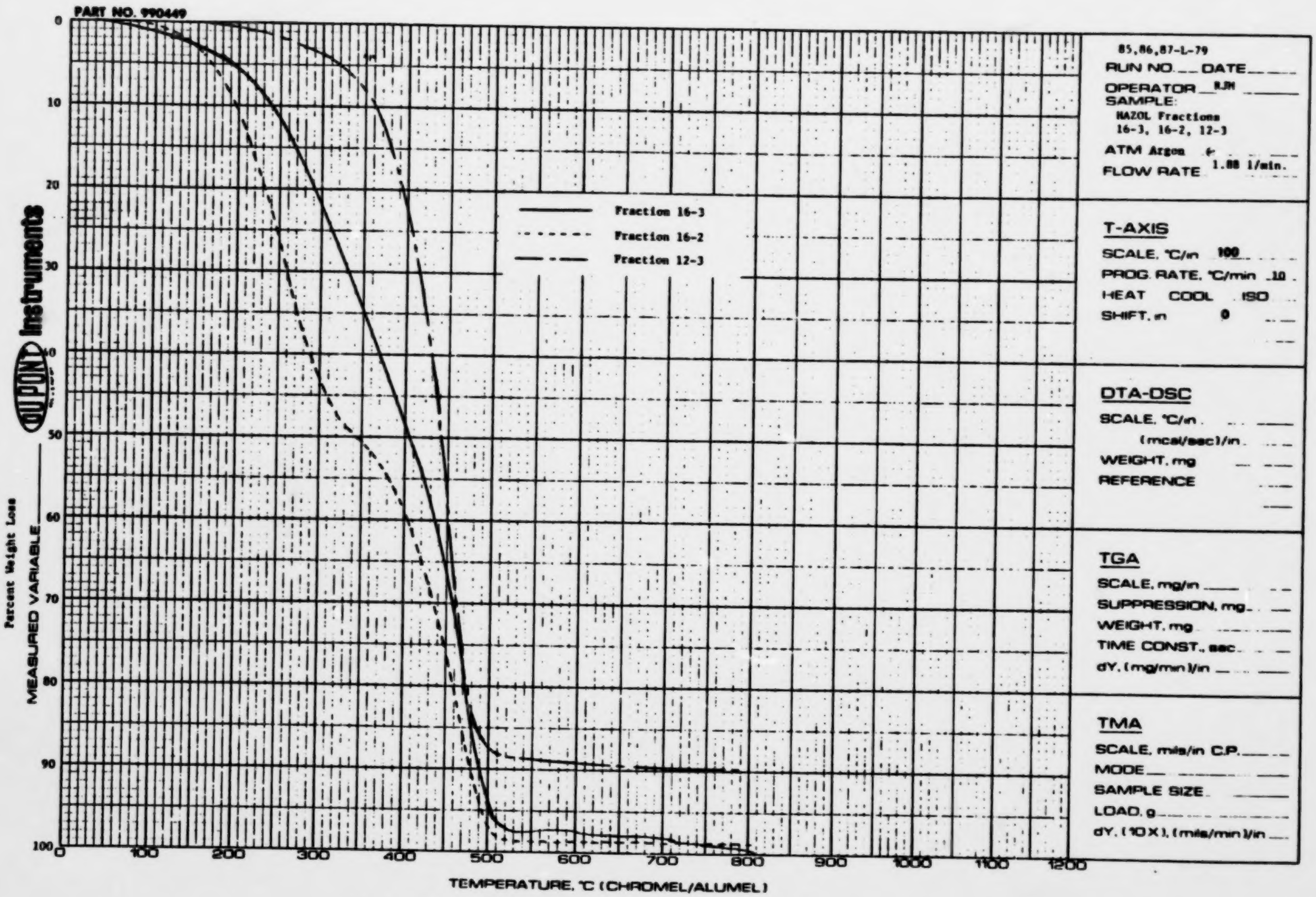


Figure 75. "Recovery" of Fraction 12-3 During Analyses



85,86,87-L-79
RUN NO. ___ DATE ___
OPERATOR RJM
SAMPLE:
HAZOL Fractions
16-3, 16-2, 12-3
ATM Argon
FLOW RATE 1.88 l/min.

T-AXIS
SCALE, °C/in 100
PROG. RATE, °C/min 10
HEAT COOL ISO
SHIFT, in 0

DTA-DSC
SCALE, °C/in ___
(mcal/sec)/in ___
WEIGHT, mg ___
REFERENCE ___

TGA
SCALE, mg/in ___
SUPPRESSION, mg ___
WEIGHT, mg ___
TIME CONST., sec ___
dY, (mg/min)/in ___

TMA
SCALE, mils/in C.P. ___
MODE ___
SAMPLE SIZE ___
LOAD, g ___
dY, (10 X), (mils/min)/in ___

Figure 76. Thermogravimetric Curves of Oil Fractions

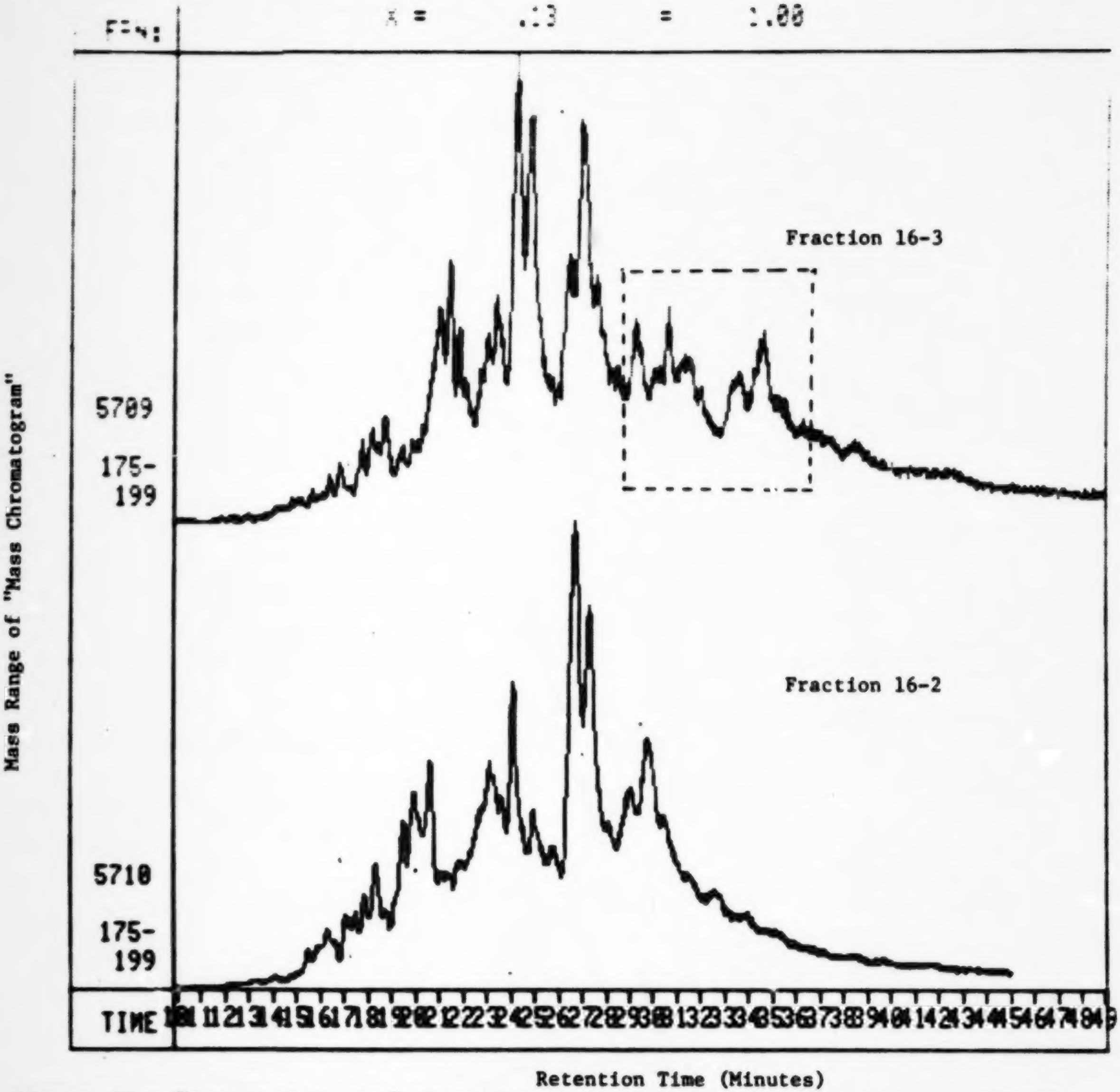


Figure 77. "Selected Masses" Chromatogram of Residual Oil Fractions on OV 225 Highlighting Material in One Fraction Not in the Other

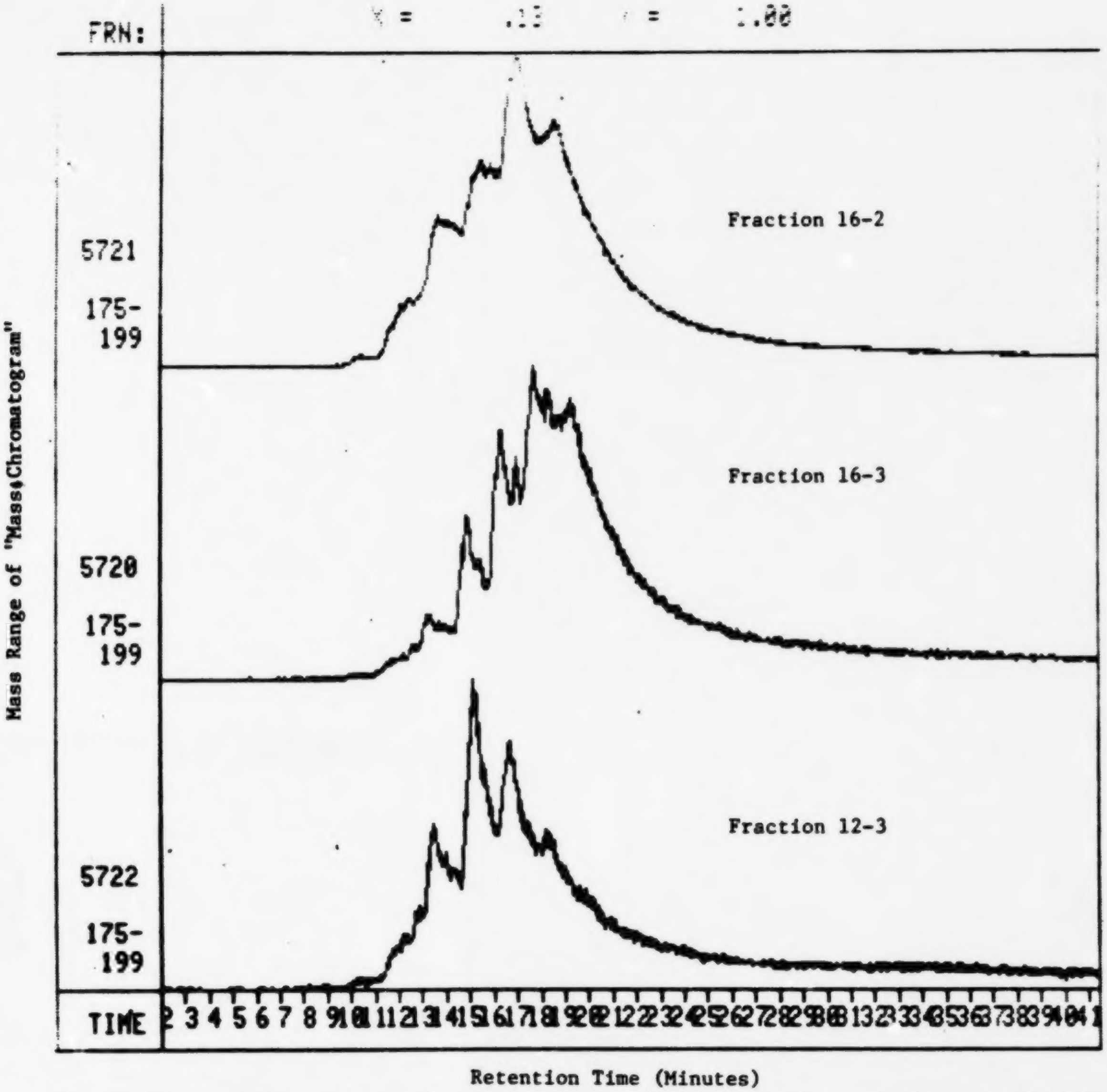


Figure 78. "Selected Masses" Chromatogram of Residual Oil Fractions on OV 101
No Differences Seen Between Fractions 16-2 and 16-3

The OV101 column data is, however, showing a unique pattern of GC peaks for 16-3 in another selected mass range (Figure 79). The pattern suggests a homologous series (not possible because of the restricted mass range), isomers (there would have to be at least seven covering a very broad boiling range) or a repeating fragment in a polymeric material. The last is the case. Running individual mass chromatograms (mass fragmentograms) on the mass range of Figure 79, data is obtained which pinpoints these peaks as being due to silicone, complete with information at mass positions due to the less abundant isotopes of silicon. The evidence for this conclusion is given in Figures 80 and 81. The silicone is being injected with the sample and is not due to GC column bleed. It may be used in processing the crude oil. The value of the data handling technique developed here lies in what it led to as reported below.

Individual mass chromatograms were run in the mass range 140 to 320 (181 chromatograms) for the data from OV225 column analyses. This was done for all three fractions (a total of 543 chromatograms) with one "page" of data showing comparisons among 16-2, 16-3 and 12-3. It was demonstrated that Fraction 16-3 contained material of molecular weight 180 that was not present in Fraction 16-2 or 12-3 and that, further, this was representative of a probable homologous series extending to mass 194 and 208 and (possibly) to 166 and 222. None of this "series" was represented in Fraction 16-2 (see Figure 82). If the bio-activity of Fraction 16-3 resides in the portion passing through the OV225 column, the materials "discovered" by the above effort would be prime candidates as causative factors.

An attempt was made to obtain more data from the above 500 mass chromatograms - in regions where GC resolution was poor. The computer program providing the individual mass chromatograms also lists the

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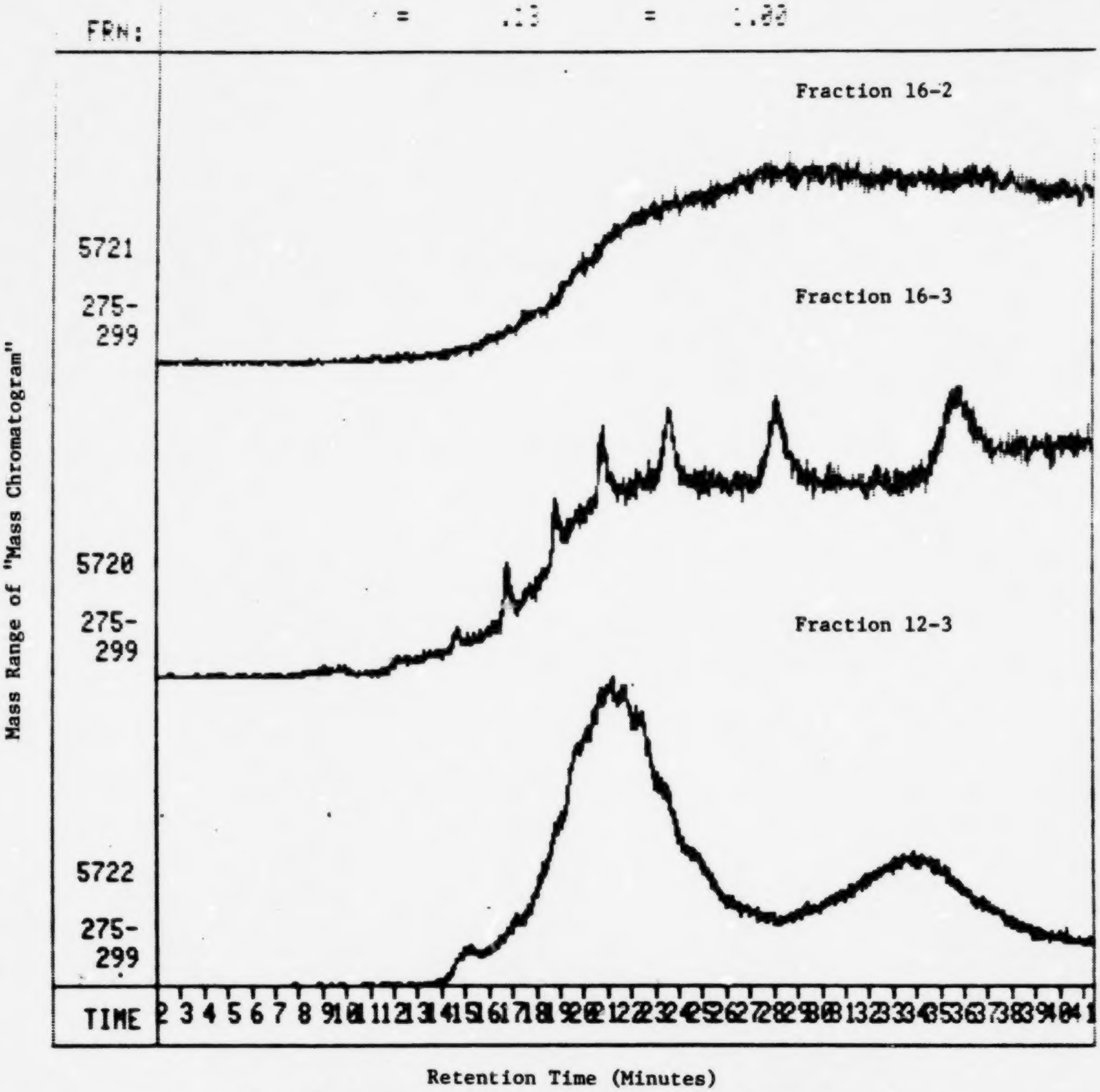

Figure 79. "Selected Masses" Chromatogram of Oil Fractions on OV 101 - Masses 275-299

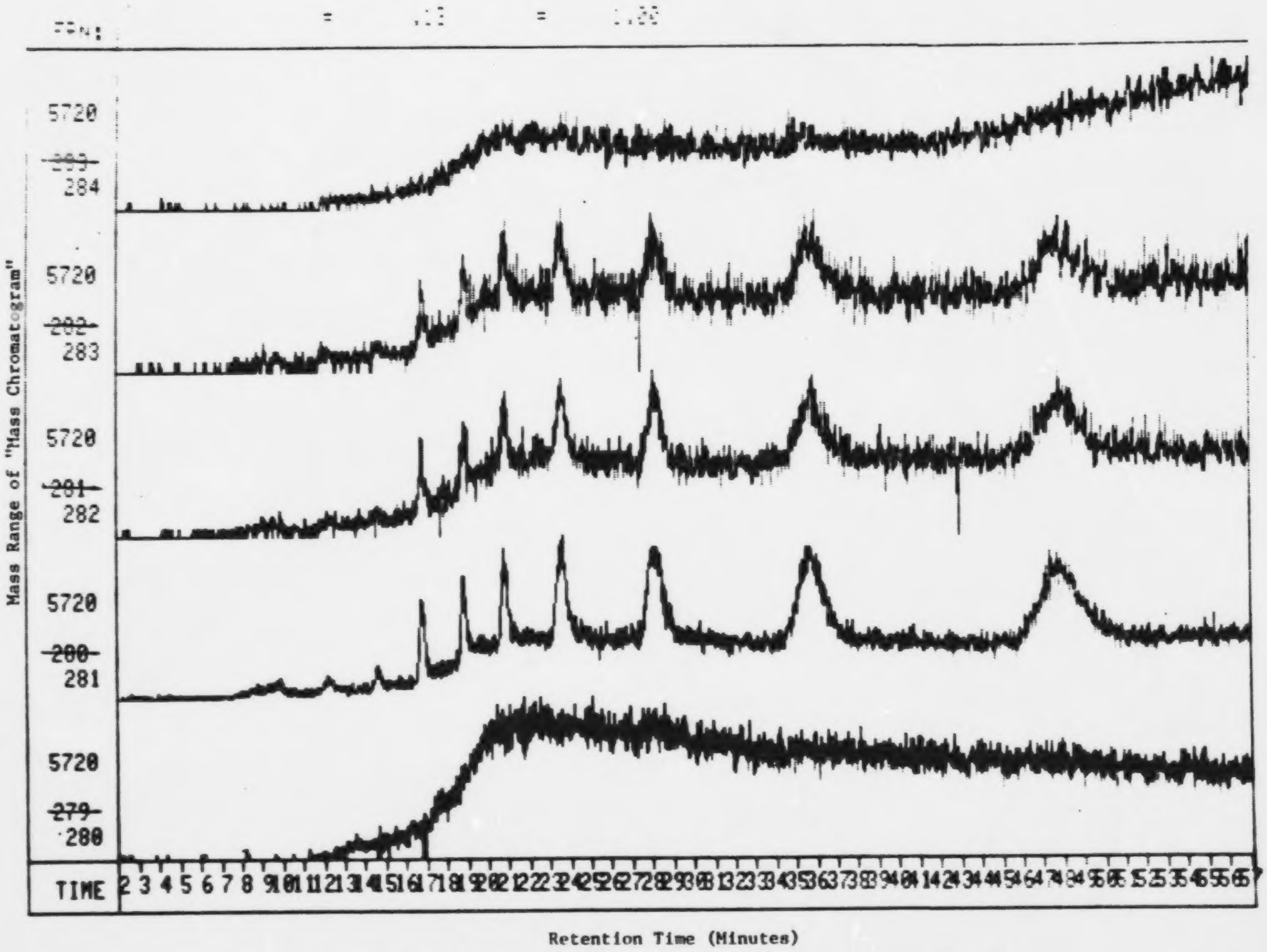

Figure 80. Pattern of GC Peaks of Masses 281, 282 and 283 in Fraction 16-3 (Mass Fragmentograms)

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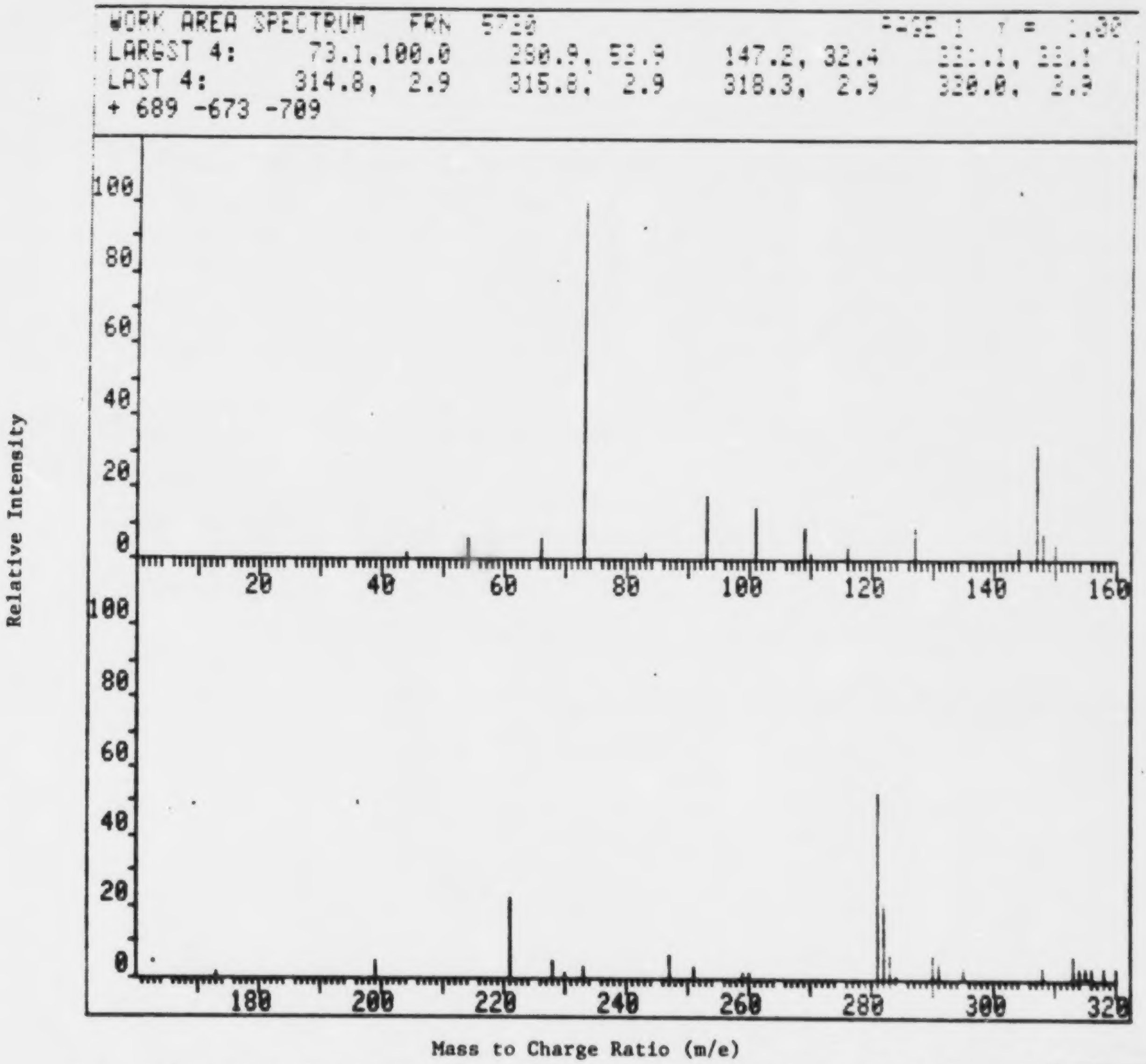

```
WORK AREA SPECTRUM   FRN  5720                              PAGE 1   Y =  1.00
LARGST 4:      73.1,100.0    280.9, 52.9    147.2, 32.4    221.1, 22.1
LAST 4:       314.8,  2.9    315.8,  2.9    318.3,  2.9    320.0,  2.9
+ 689 -673 -709
```

Figure 81. Mass Spectrum that is Typical of Each of the Peaks in Figures 79 and 80

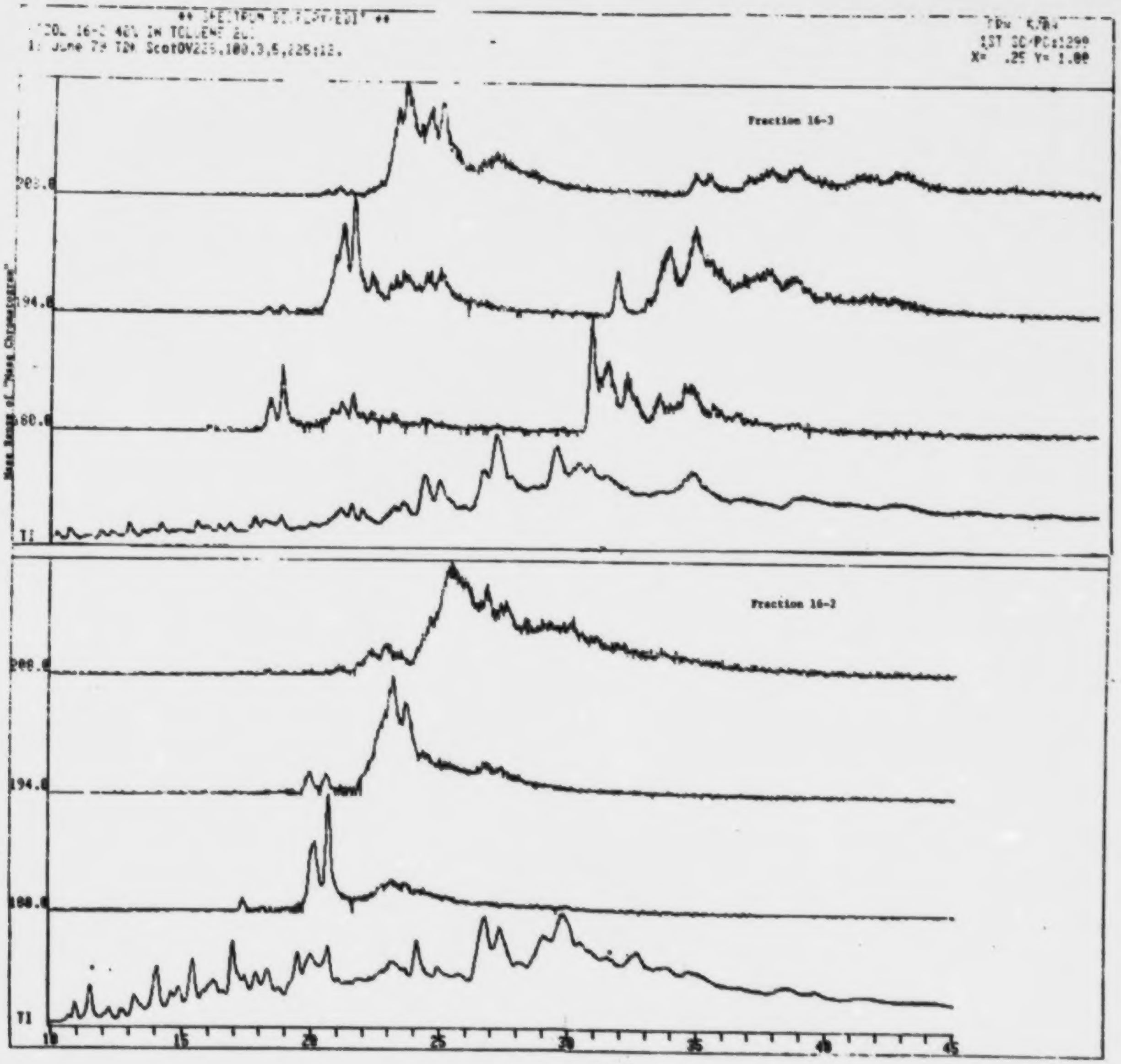

Figure 82. Comparison of Mass Fragmentograms for Fractions 16-3 and 16-2

total number of ions of the given mass present in the complete GC trace. This value can be plotted as in Figure 83. The important features of Figure 83 are at positions where:

1. There is an excess of ions in 16-3 as compared to 16-2. This occurs at masses 215, 230 and 234 (marked by dashed arrows in Figure 83). Mass 215 is probably redundant since it could represent a molecule of mass 230 losing a methyl group. This would indicate material of molecular weight 230 and 234 as being novel to or more abundant in Fraction 16-3 as compared to 16-2.

2. Positions where there is a decidedly more positive slope for 16-3 data than for 16-2 data. This occurs most obviously at masses 166, 180, 194, 208 and 222 (solid arrows). This confirms the data of previous Figure 82.

Column Chromatography Pre-Separation Fractions of DMSO Partitioned Sample

As reported above dimethysulfoxide (DMSO) extraction is very effective in partitioning residual fuel oils into primarily aromatic and primarily aliphatic fractions. This process operates on the as-received sample differently than our 7-fraction column chromatography sample pre-separation. Our work has shown that use of dimethyl sulfoxide (DMSO) partitioning combined with column chromatography pre-separation leads to sample information not previously available.

The DMSO insolubles (85% of sample) and DMSO solubles (15% of sample) of oil #16, as each separated into our 7 pre-fractions by column chromatography, have been examined by gas chromatography/mass spectroscopy (the fractions from the DMSO insolubles form the 16i series of 7 fractions; from DMSO solubles, the 16s series). Quantitative data on the recovery of the various fractions from the column are shown in Figure 84. To better illustrate differences among the samples as

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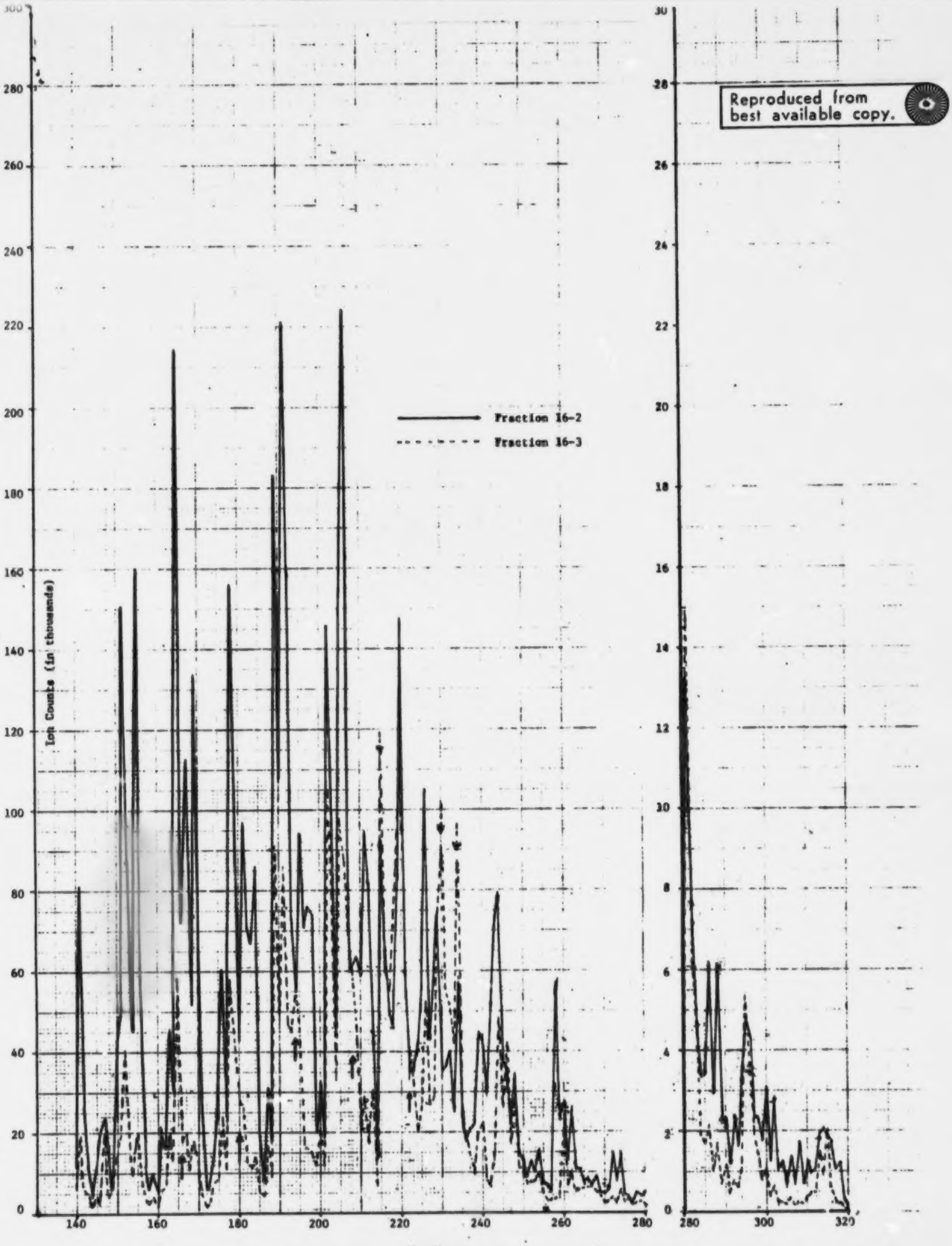


Figure 83. Ion Abundances at Each Unit Mass for Fractions 16-2 and 16-3 as Recovered from SCOT OV 225 Column

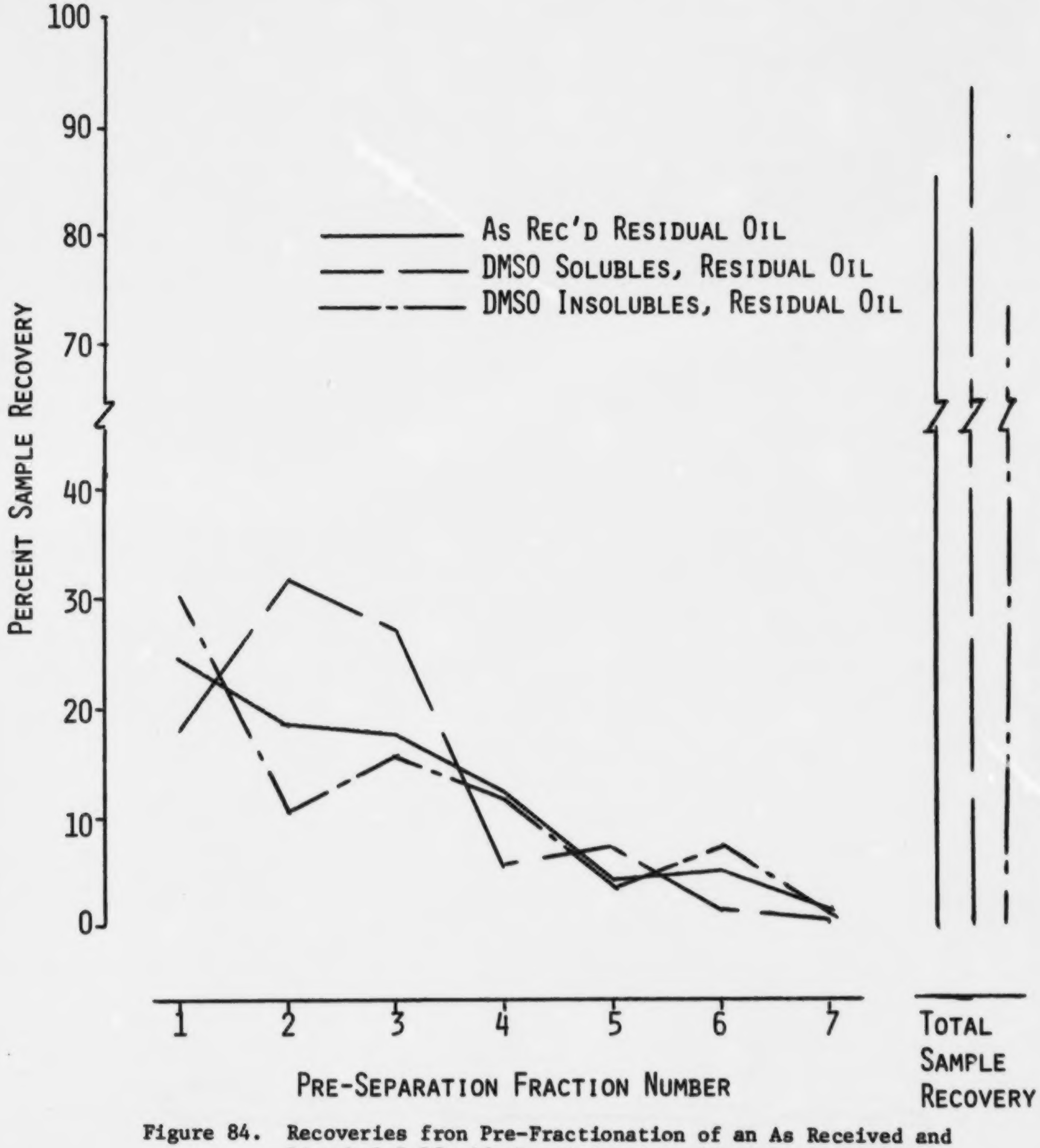


Figure 84. Recoveries fron Pre-Fractionation of an As Received and Dimethyl Sulfoxide-Partitioned Residual Fuel Oil #16

obtained, Figure 84 does not reflect the 15% solubility/85% insolubility of oil #16 in DMSO. The figure does show:

1. The DMSO partitioning is a different process than our column chromatography separation.
2. It is logical to expect combined DMSO partitioning/column separation to lead to some degree of simplification of sample analyses.

Generally speaking, the 16i series of fractions consists primarily of non-aromatic types of organic compounds. This is exemplified in Figure 85 where the total ion gas chromatograms of Fraction 2 - as received, DMSO insoluble and DMSO soluble - of residual fuel oil #16 are depicted. The pattern of Fraction 2 from DMSO insolubles (middle trace in Figure 85) shows the periodic peak pattern associated with aliphatic organic homologues. This is confirmed by mass spectroscopy. The GC peaks in Fraction 2 of DMSO solubles (top trace in Figure 85) are shown by mass spectra to be due to polycyclics. An interesting feature of the data of Figure 85 is the apparent loss of signal due to material responsible for peaks as in the inscribed portion of the bottom trace (as received oil #16 fraction) as compared to the other Fractions 2. GC/MS analysis of all 14 fractions (i.e. - 16i and 16s series) shows that DMSO separation primarily into aliphatic and aromatic species is general. There has therefore been no extensive chemical examination of the 16i series. As reported above, bioassay indicates these are not of environmental concern. Fraction 1, 16s, is particularly interesting because it is expected to be primarily aliphatic hydrocarbons. But DMSO is not a solvent for such material and Figure 84 tells us that an appreciable portion of the DMSO solubles of oil #16 elutes off our column as Fraction 1.

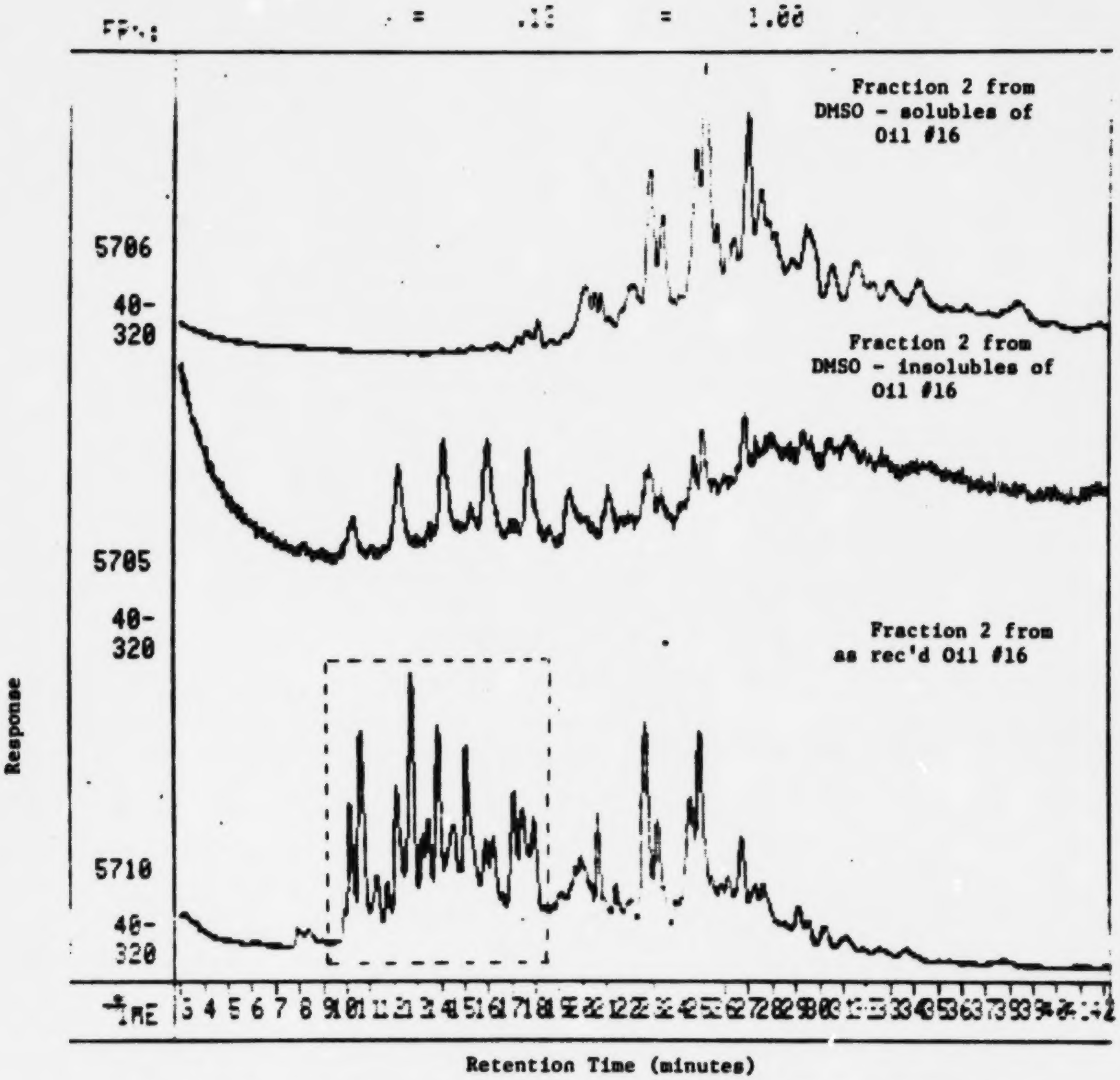


Figure 85. Total Ion Gas Chromatographic Traces Comparing Various Fractions #2 from Residual Fuel Oil Sample #16

Total ion chromatograms by GC/MS of all the "Fraction 1" samples are shown in Figure 86. The samples derived from as-received and DMSO insoluble oil #16 are primarily aliphatic hydrocarbons. The material in Fraction 1, DMSO soluble, oil #16, however, is primarily polycyclic aromatics starting with homologues of naphthalene. Such material would be expected off our column as part of Fraction 2. Such "fraction skipping" behavior of material has been noted before. This reinforces our contention that the nature of the sample onto column can affect the elution of various components - at least in the case of relatively large sample loads such as we have to use.

Also, mention has been made above concerning the ability of material to show up in more than one fraction of our 7-fraction sample pre-separation. Usually this "carry over" represents only a minor portion of a particular substance and would probably be equated normally to compound retention times that cause some premature or tardy elution with a given solvent system. There is indication here that more than this may be involved. Figure 87 offers good evidence. Some of the "missing" material from a Fraction 2, as discussed above, is now seen to elute in a Fraction 1 (See the inscribed hexagons of Figure 87). Study of this type of data suggests the following possibilities in terms of our types of samples:

1. The general chemical nature of the sample onto column in column chromatography will affect the polarity of a given fraction eluted off column.
2. Where bias as above is introduced, the early solvent system will contain lower molecular weight/simpler ring systems than the later solvent systems. The change is systematic and includes non-proportionate but patterned partitioning of substances occurring in both "earlier" and "later" solvent systems (see inscribed rectangle areas of Figure 87).

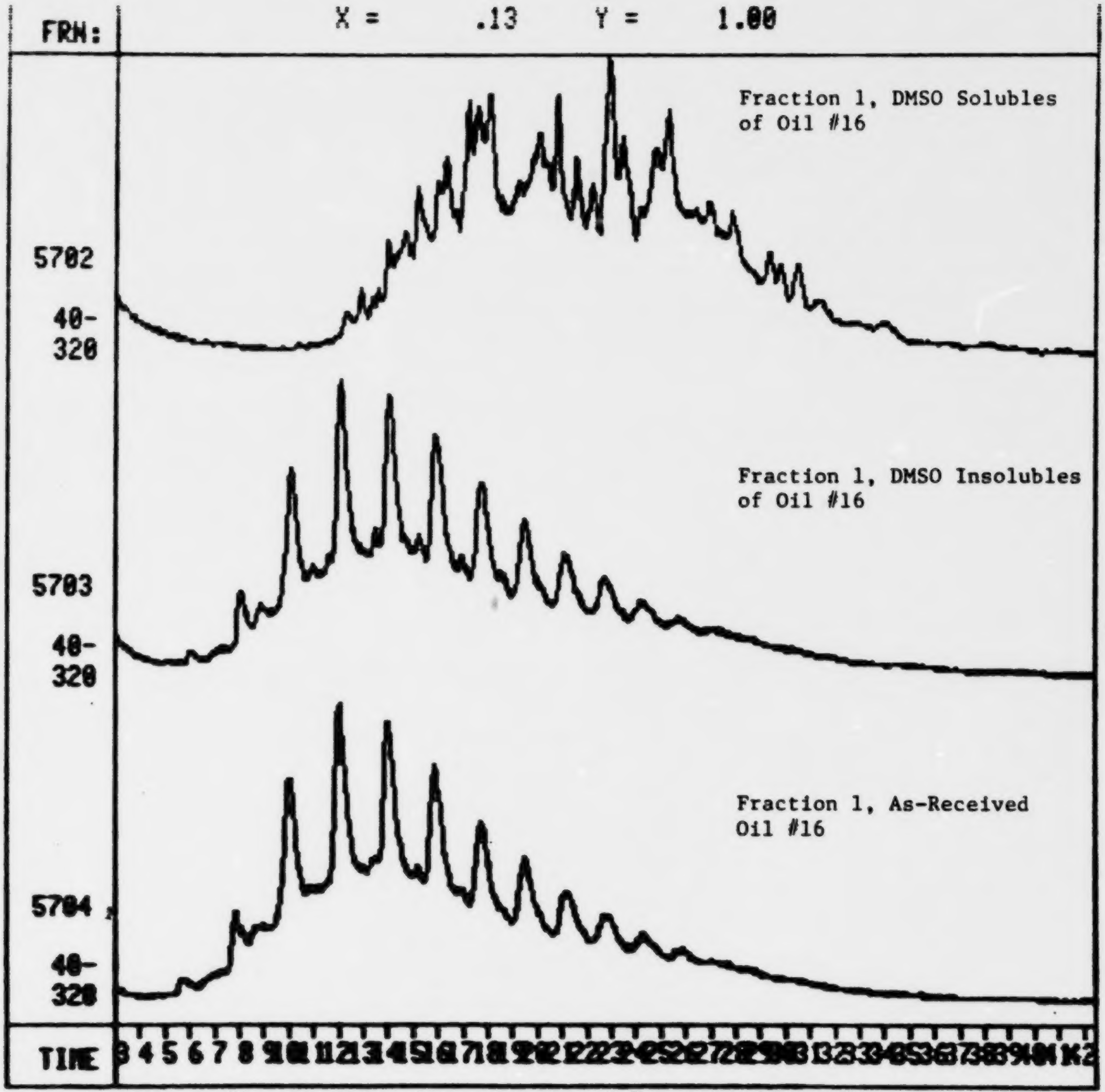


Figure 86. Total Ion Chromatograms of "Fraction 1" Samples Derived from Oil #16

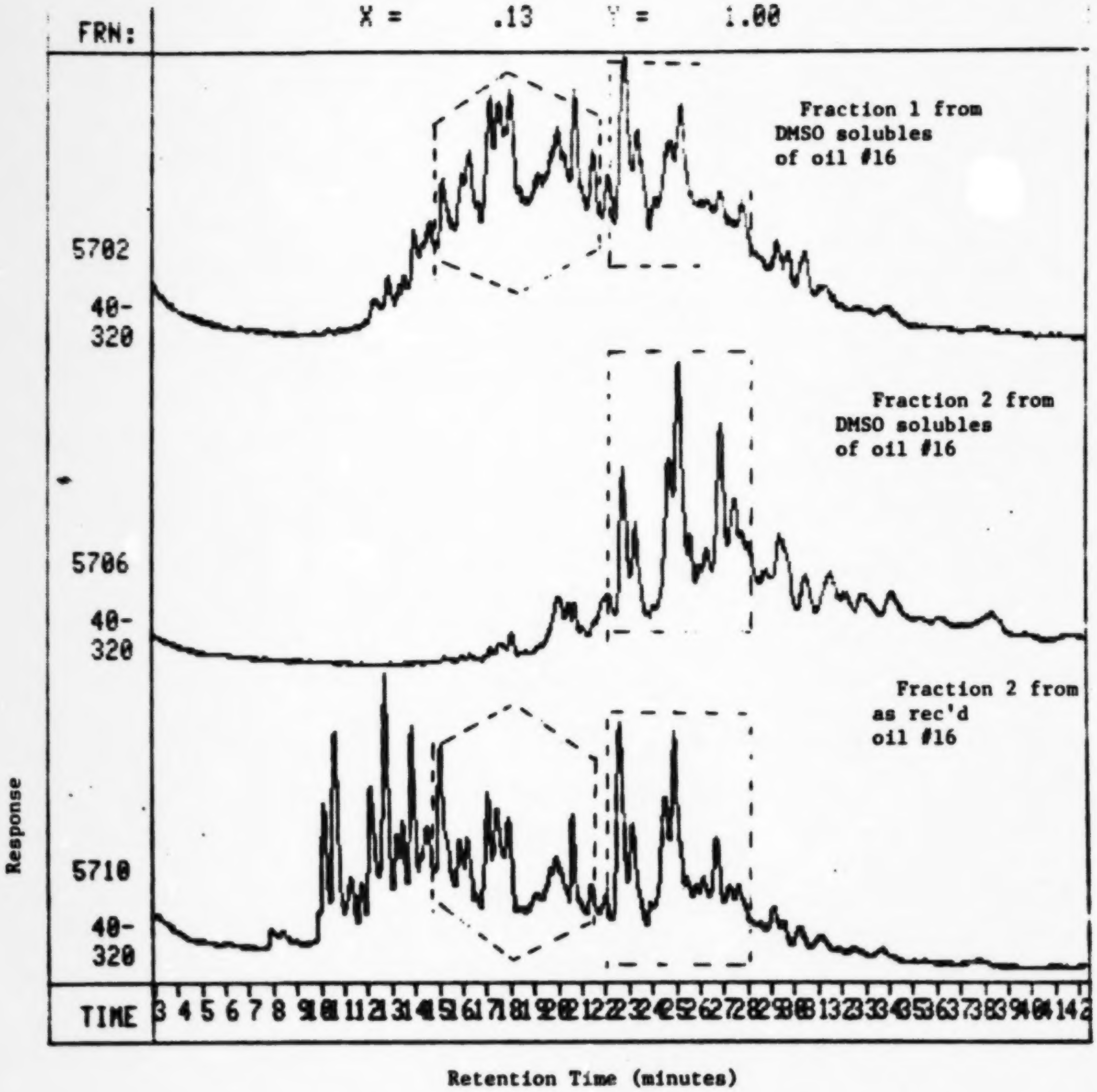


Figure 87. Evidence for Occurrence of a Given Material in Different Fractions of a Sample

3. The process of component elution off column may itself change the chemical nature of remaining sample enough to cause changes in eluants. Some of the sample components are seen in several of the eluted fractions of a given sample.

4. Any of the above observations may be mitigated by the very low analytical volatility through the GC/MS of these types of samples. Effects we are noting above may be minor in terms of the bulk of the fractions.

5. Removal of aliphatic "bulk" from a sample through DMSO partitioning (or its initial scarcity in a sample) may lead to eventual loss of more volatile sample material such as methyl and/or ethyl naphthalenes. These materials may not be assayed properly based on how much aliphatic material there is in a sample to act as a fixative.

A similar condition highlighted by this work with the 16s series of fractions may be a special characteristic of our unique column pre-separation material. Materials found in Fraction 16-1s (probably dibenzothiophene and its homologues) are also found in Fraction 16-6s and materials found in Fraction 16-2s (probably benzanthracene, benzophenanthrene, chrysene, or triphenylene and homologues) are also found in 16-7s. As discussed above, our column material contains sodium sulfate to increase its polarity. The original thought was that once a sample was eluted with a solvent system, the column could be washed with water, dried, and re-eluted. Initial work indicated that not much additional material was recovered in this fashion and it was decided that the return/effort ratio was too small for this contract. The column composition was retained, however, since it gave good separations and recoveries even without the water wash. On repeated use with our current elution solvent system, it became clear that our Fraction #5 solvent (methanol) was washing out sodium sulfate, effectively changing the polarity of the column as per original intent. In terms of the analytically volatile portions (which are only a very small part), Fractions 6s and 7s resemble the higher molecular weight/more complex ring systems found in Fractions 1s and 2s (see Figure 88). This effect could be potentially important for at least three reasons:

1. It could be a means of obtaining the more complex polycylic hydrocarbons and heterocyclics in a more concentrated form.
2. The "non-volatile" (major) portions of Fractions 6s and 7s should also reflect this effect and may indeed have novel materials as compared to non-volatiles from Fractions 1s and 2s.
3. Most importantly, because of increased complexity, Fractions 6s and 7s may concentrate any bioactivity potentially present in Fractions 1s and 2s.

Due to the low volatility of samples, we are interested in categorizing fractions from, rather than characterizing compounds in, residual fuel oils. As indicated our effort is directed at the 16s rather than the 16i series of column chromatography fractions. An ideal way to perform this chore is to utilize the GC/MS computer capability to reconstruct individual ion chromatograms and to plot 5 such chromatograms in comparison on a single page. An ion grid that can be used to detect all homologous series within a specified molecular weight range is shown in Figure 89. (Figure 89 covers the MW range 137 to 276. For Fractions 3 through 7 a similar grid, MW range 166 to 304, was used). In Figure 89, a vertical column produces 2 pages of ion chromatograms, each chromatogram differing from the previous and following by 14 mass units. This mass gap corresponds to molecular weight differences among the members of a homologous series. If a column of "odd" mass numbers is used, the data will show a homologous series with a parent compound having an odd number of nitrogen atoms in its molecule. (Care must be taken, however, that the odd mass does not represent a molecule minus, for example, a methyl group.) Applying

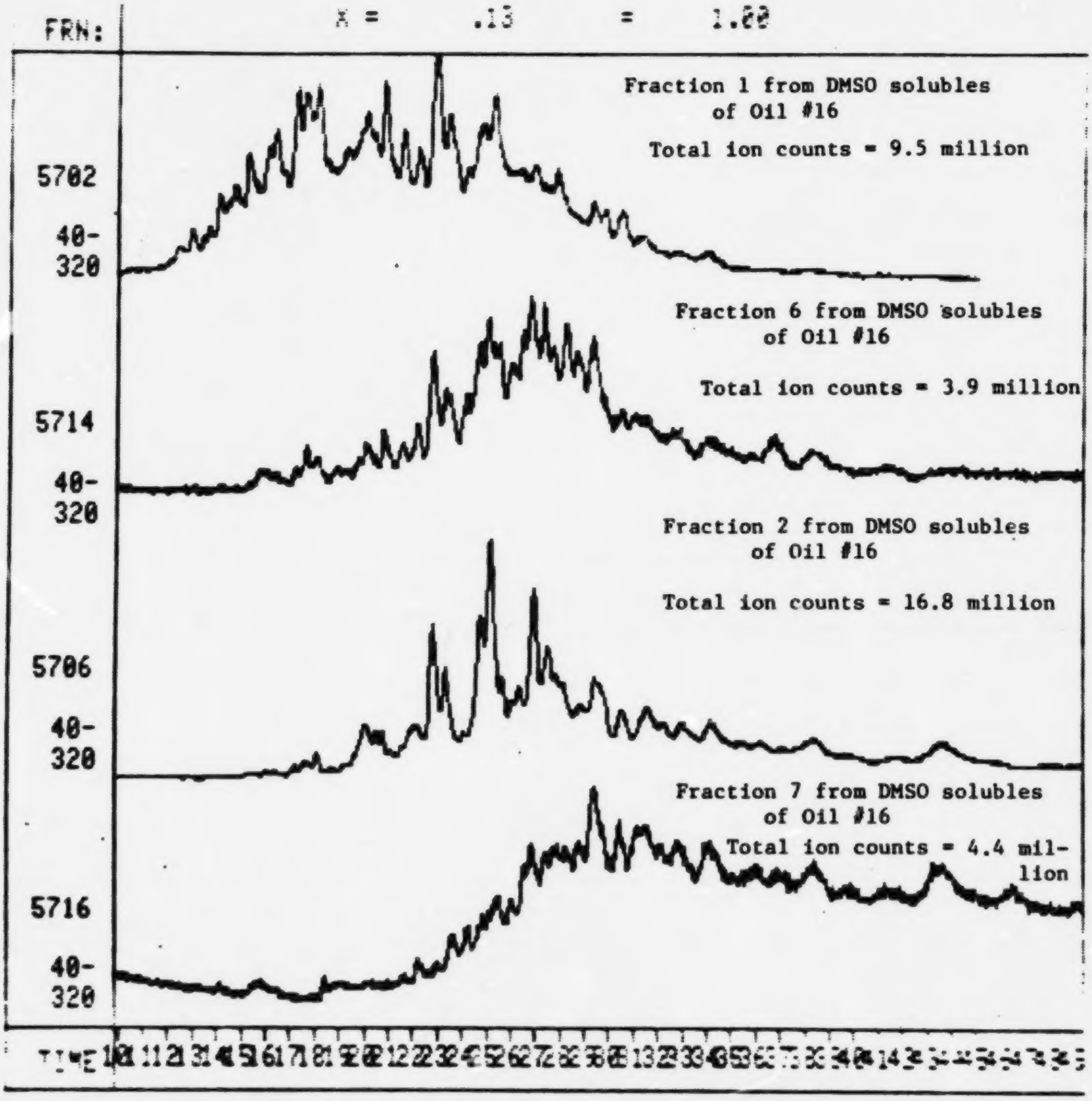


Figure 88. Total Ion Chromatograms of Oil Fractions Having Some of the Same Components

137	138	139	140	141	142	143	144	145	146	147	148	149	150
151	152	153	154	155	156	157	158	159	160	161	162	163	164
165	166	167	168	169	170	171	172	173	174	175	176	177	178
179	180	181	182	183	184	185	186	187	188	189	190	191	192
193	194	195	196	197	198	199	200	201	202	203	204	205	206
207	208	209	210	211	212	213	214	215	216	217	218	219	220
221	222	223	224	225	226	227	228	229	230	231	232	233	234
235	236	237	238	239	240	241	242	243	244	245	246	247	248
249	250	251	252	253	254	255	256	257	258	259	260	261	262
263	264	265	266	267	268	269	270	271	272	273	274	275	276

Figure 89. Ion Chromatogram "Grid" for Detecting Homologous Series in the Molecular Weight Range 137 to 276

a grid such as in Figure 89 to the 7 fractions of the 16s series is tedious (the work here required several days). The results as exemplified in Figure 90 justify the effort. Figure 90 is data as obtained from a grid such as in Figure 89. Added is a total ion chromatogram of the fraction (bottom trace) to show sample complexity in relation to individual ion chromatograms. Added also is a mass 153 ion chromatogram to show that the homologous series does indeed start at mass 167 (i.e., 14 mass units above 153). Deleted are individual ion chromatograms at masses 251, 265, 279, and 293 which do not have any completely clear indications of extension of the homologous series evidenced in Figure 90. The data of the figure show:

1. The ion chromatogram at mass 167 shows a single peak. No compound isomers are indicated so that the material of the peak is most likely the parent for the homologous series. (Based on mass spectra, it is also probably carbazole.)
2. The peaks of all ion chromatograms above 167 progress systematically to higher column retention time (i.e. - higher boiling point). This is indication (additional to the 14 mass unit intervals) that a homologous series is represented.
3. The number of peaks in a mass chromatogram is also the minimum number of isomers of the homologue in the fraction.
4. The homologous series present in the sample extends in molecular weight from 167 through 237 which corresponds to a final 5 carbons as alkyl groups substituted on the parent compound. (These are probably mostly if not exclusively methyl and/or ethyl groups.)

Data analyses as above involving the analytically volatile portions of fractions 3s through 7s from oil #16 lead to the data of Table 34. The major concern of this work is, of course, possible environmental impact of sample components, and, if any additional work is done in the future,

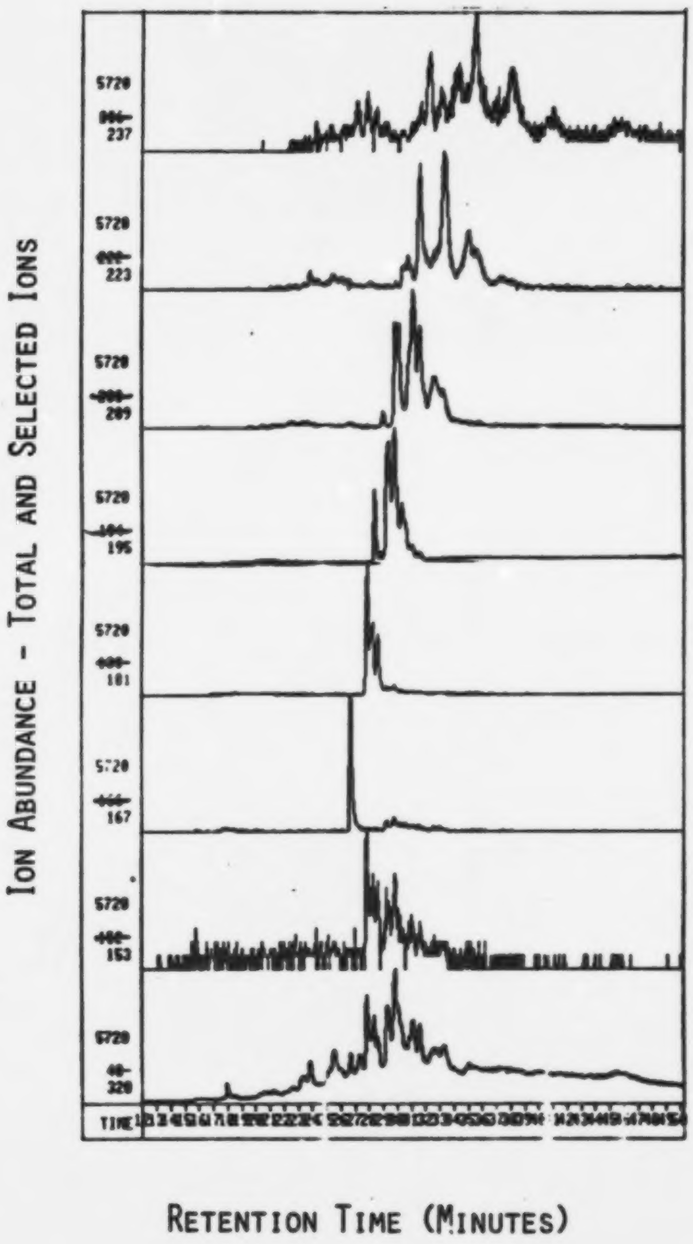
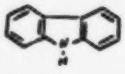
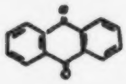
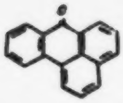
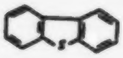
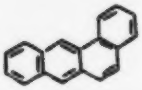
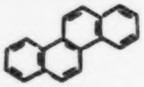
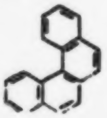


Figure 90. Evidence for a Homologous Series in Fraction 16-3 (Gas Chromatographic Traces)

Table 34

TYPES OF ORGANICS PROMINENT IN THE VARIOUS FRACTIONS OF OIL #16, DMSO SOLUBLES, VOLATILE PORTION

FRACTION	COMPONENT		APPARENT HOMOLOGUES AT M.W.:					
16-3a	M.W. 167 (Carbazole)		181	195	209	223	237	
16-4a	M.W. 208 (Anthraquinone)		222	236	250			
	and							
	M.W. 230 (Benzanthrone)		244	258				
16-5a	(?)							
16-6a	M.W. 184 (Dibenzothiophene)		198	212	226	240	254	268
16-7a	M.W. 228 (a four ring hydrocarbon - examples at right)	  	242	256	270			

model compounds as categorized in Table 34 should be bio-evaluated. It will be noted that Fraction 5s has very little "analytically volatile" material and that the types of compounds in fractions 3s and 4s may be of more interest than those in 6s and 7s. Fractions 1s and 2s have not been as exhaustively evaluated but they appear to contain at least the additional homologous series:

Table 35

APPARENT HOMOLOGOUS SERIES IN FRACTIONS 16-1s AND 16-2s

M.W. of Parent Compound	M.W. of Indicated Homologues						
166	180	194	208	222	236	250	
168	182	196	210	224	238	252	
178	192	206	220	234	248	262	276
202	216	230	244				
?	236	250	264				
234	248	262					

One apparent fact that may not be evident just from the data of Tables 34 and 35 is that, at least in terms of volatiles, oil #16 consists primarily of certain "parent" compounds and then many methyl and ethyl homologues of them.

Sample Volatiles

Volatiles from 3 oil samples were briefly examined by GC/MS. The bioassay of these volatiles is reported above and all checked negative.

The apparatus of Figure 91 was used to collect these volatiles. In use, the portion of the device atop the oven was covered with a fabric-type thermal insulator. The three residual fuel oil samples were treated in the apparatus for four hours at 110°C.

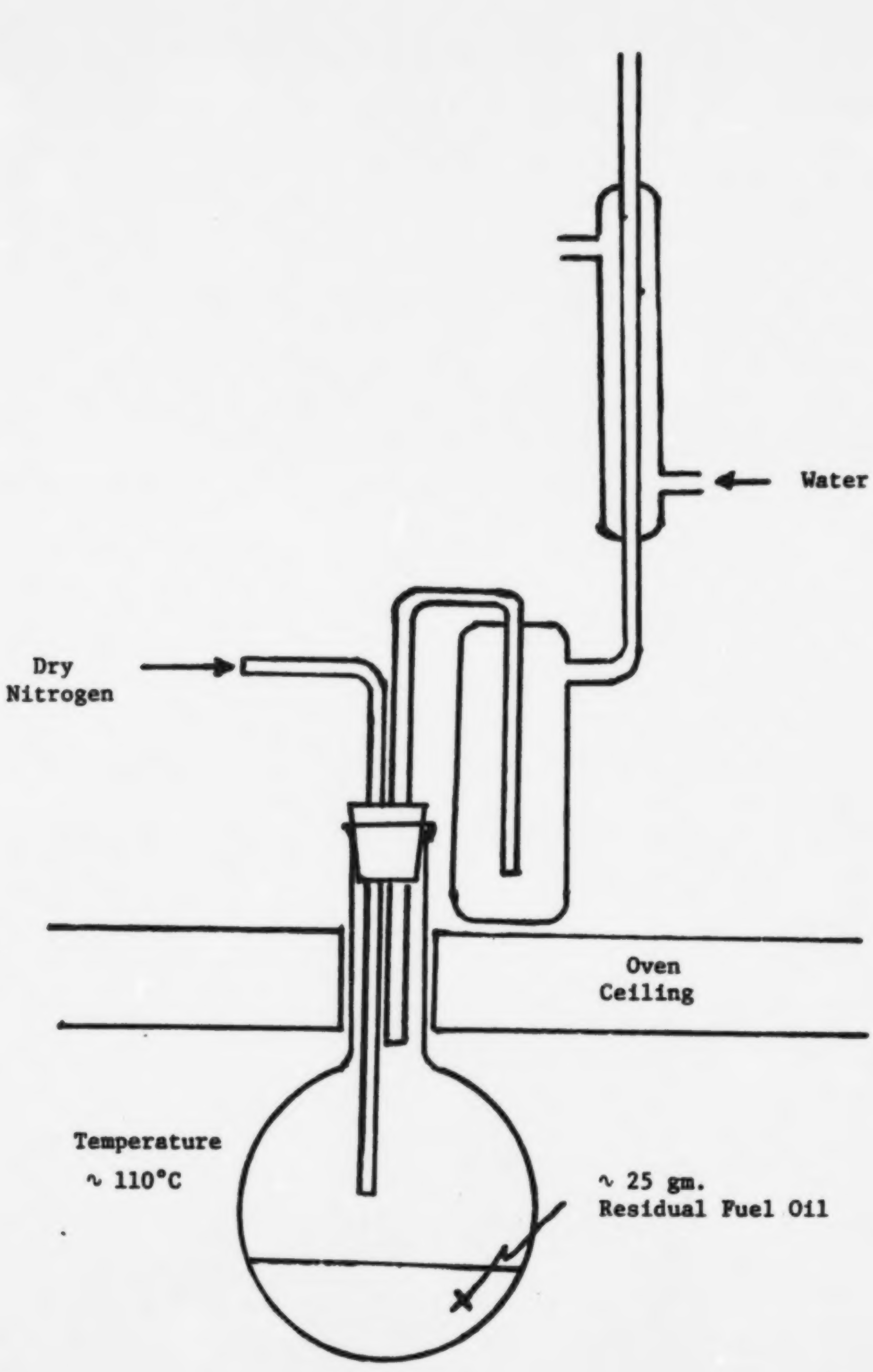

Water
Dry Nitrogen
Oven Ceiling
Temperature
∿ 110°C
∿ 25 gm.
Residual Fuel Oil

Figure 91. Apparatus for Recovering Volatiles from a Residual Oil

The data were as follows:

Table 36

DATA ON VOLATILES FROM SAMPLES

Oil Sample Number	Reason for Selection	Percent Weight Loss During Test
3	A mutagenic oil. Sample with most background on mutagenicity by SRI	2.7
24	"Most" mutagenic oil according to SRI	2.3
11	A non-mutagenic oil according to SRI	3.5

The greater portion of the recovered volatiles were diluted in dioxane for bioassay by Westinghouse. Portions were also subjected to GC/MS analyses. The resultant chromatograms are shown in Figure 92. In Figure 92, data from retention time about 15 minutes on represents species seen in previous work with column chromatography pre-Fraction #2. Between about 8 minutes and about 15 minutes, the compounds appear to be largely alkylated benzenes. Aliphatic compounds appear concentrated from beginning to about 8 minutes.

There is a very obvious difference in the chemical nature of volatiles from the non-mutagenic oil (top of Figure 92) as compared to the two mutagenic oils. The differences need not be associated with any supposed mutagenic activity. The major differences between volatiles reside in the first 1/3 of the chromatogram and involve at least 3 major GC peaks and several minor ones. This portion of the GC of volatiles from oil sample 24 is expanded in Figure 93.

In Figure 93, spectrum #209 is of undecane and spectrum #328 of dodecane — neither of which would be mutagenic. Spectra #272, 284 and 340 all appear to involve more than one material and are not directly related to library mass spectra. These spectra are included here as Figures 94 through 96.

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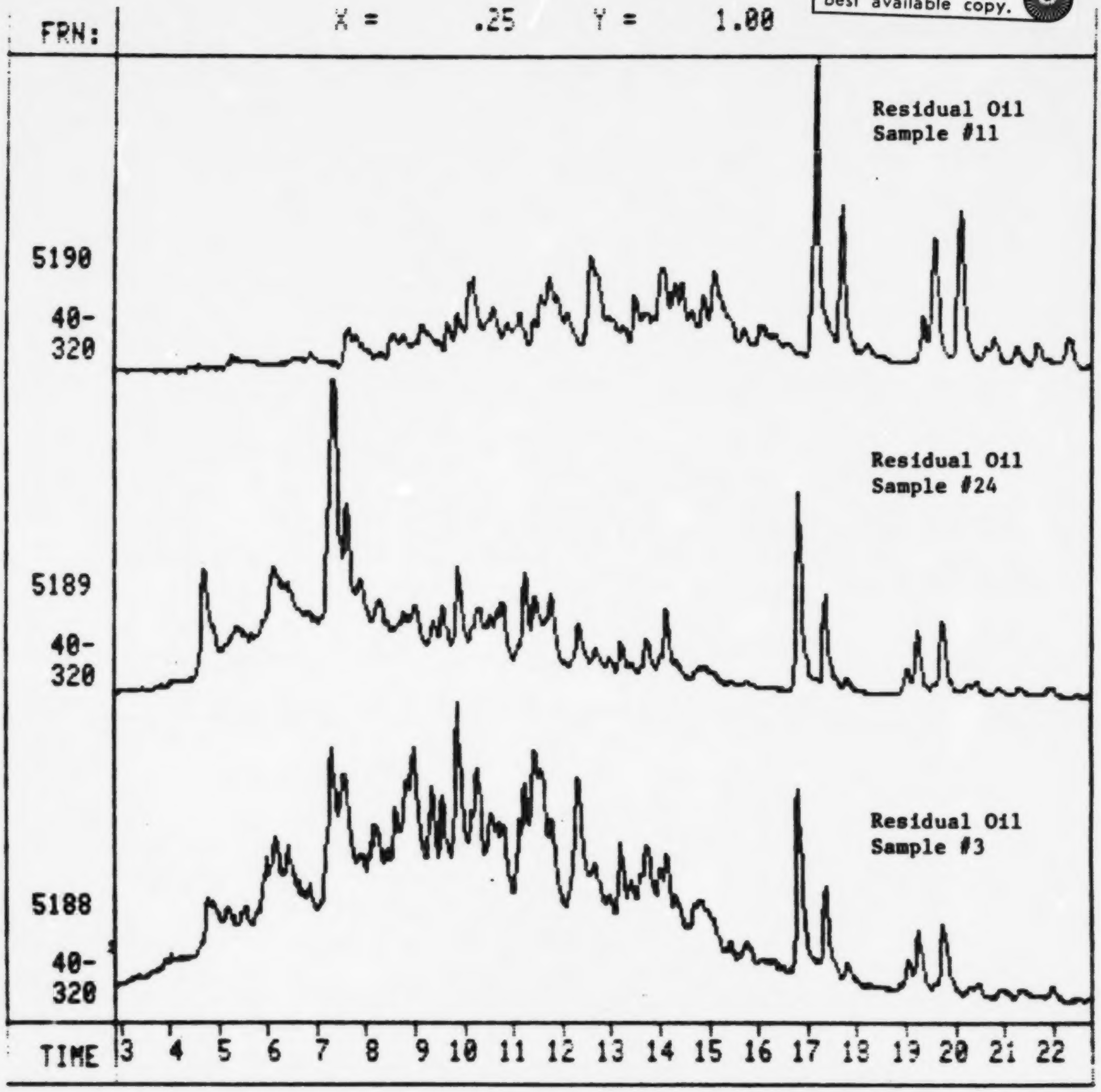


Figure 92. Gas Chromatograms of Volatiles from Residual Fuel Oils

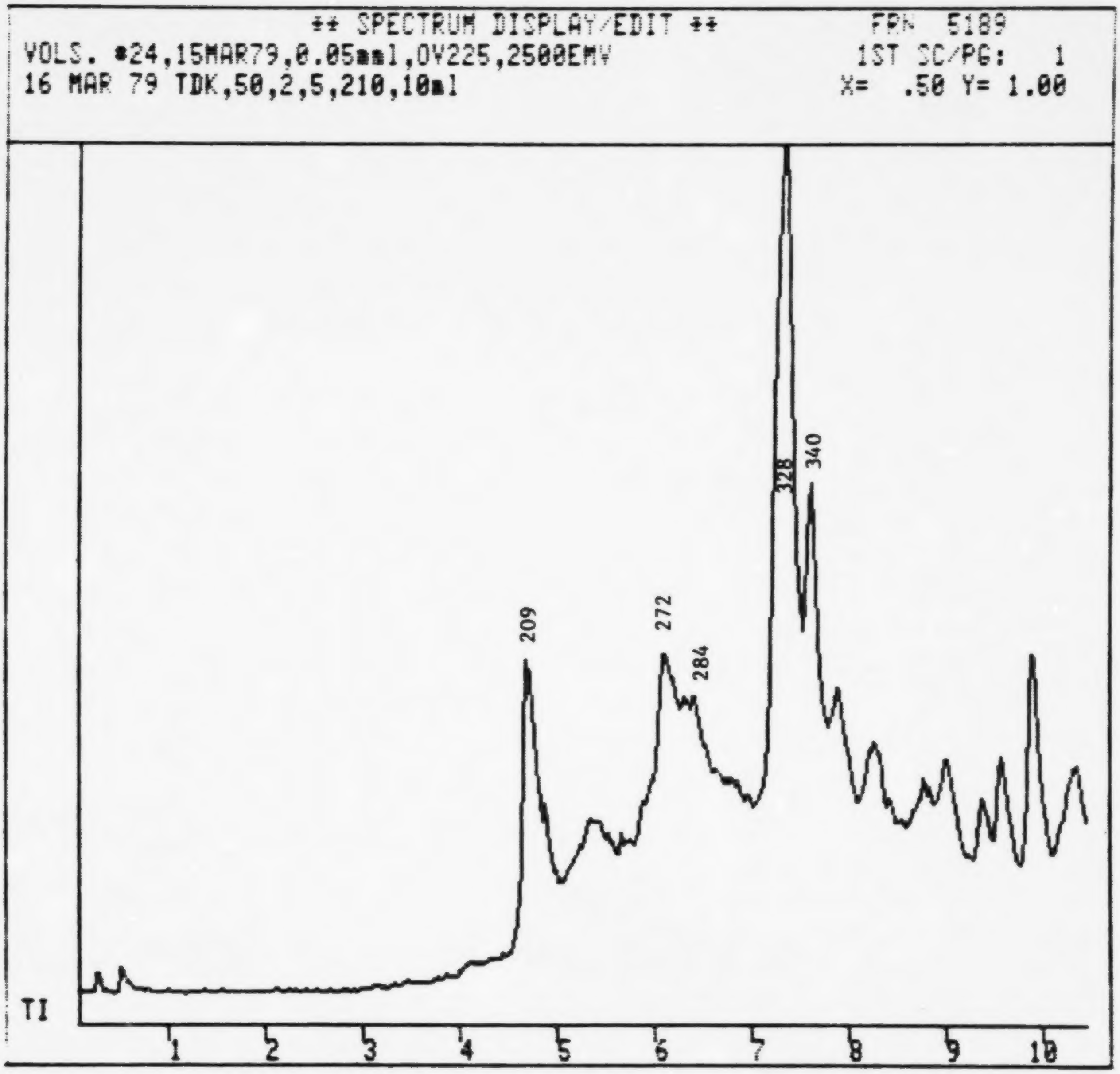
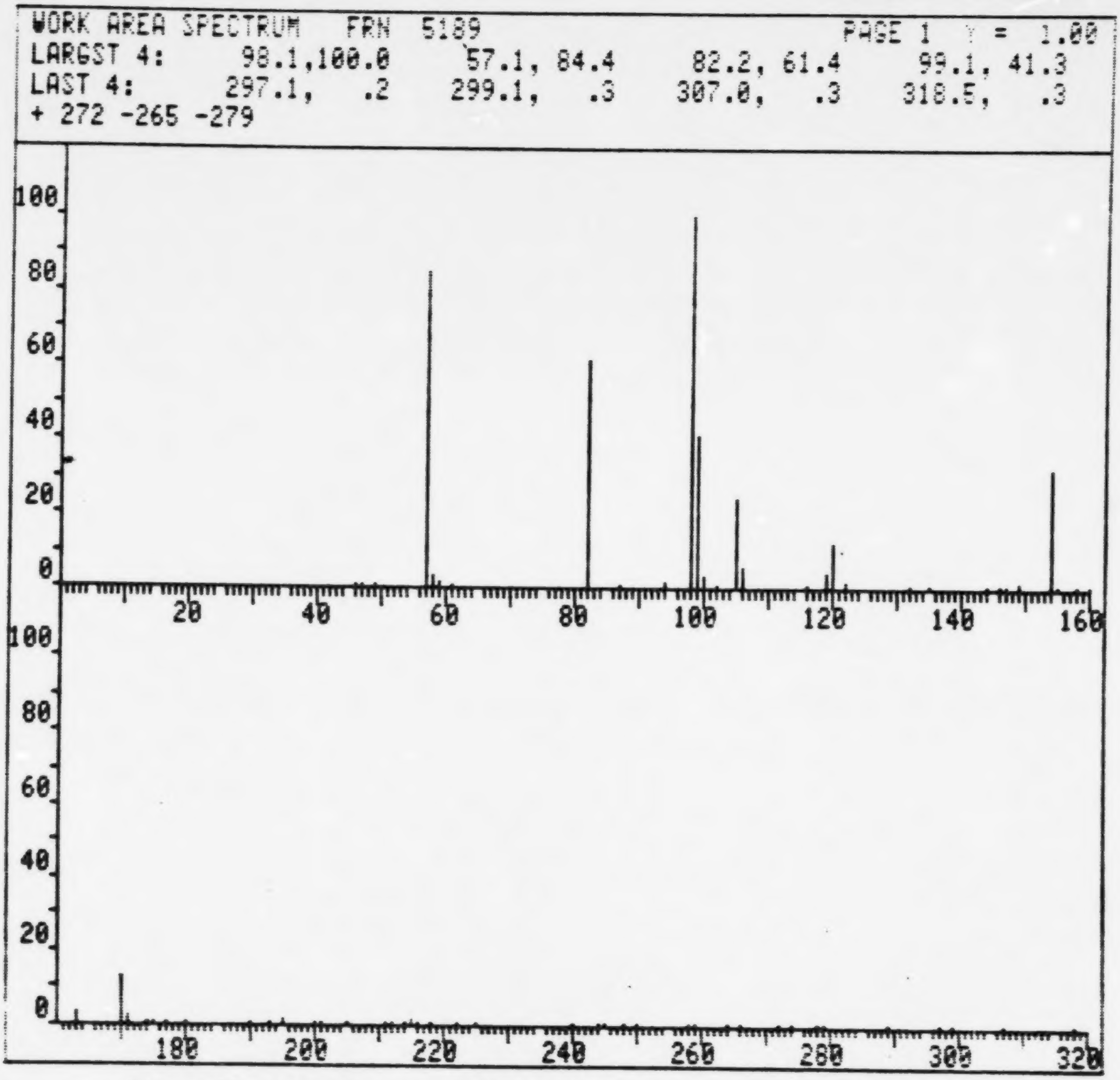


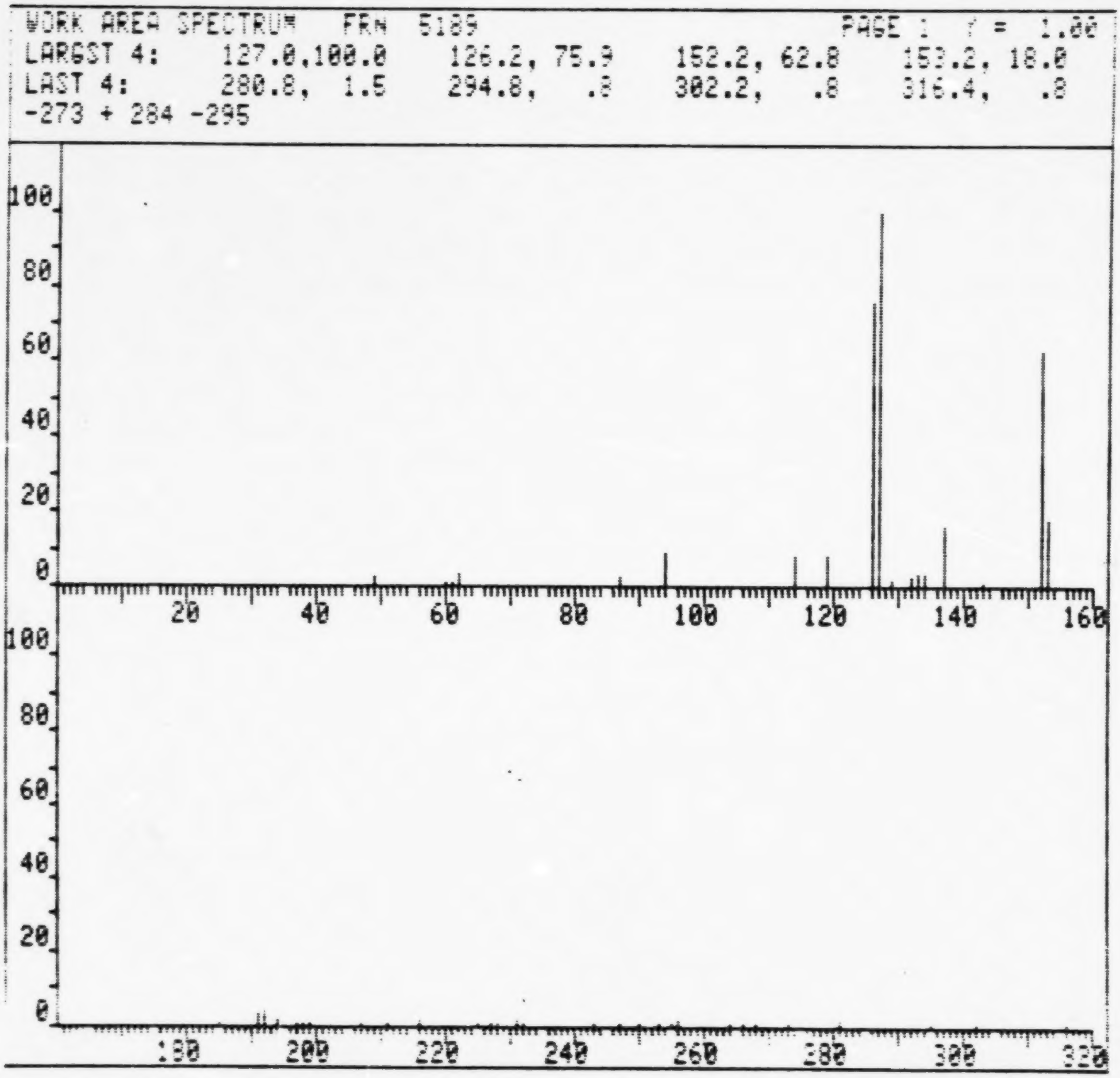
Figure 93. Initial Portion of GC Trace of Volatiles from Oil Sample #24

Reproduced from
best available copy.



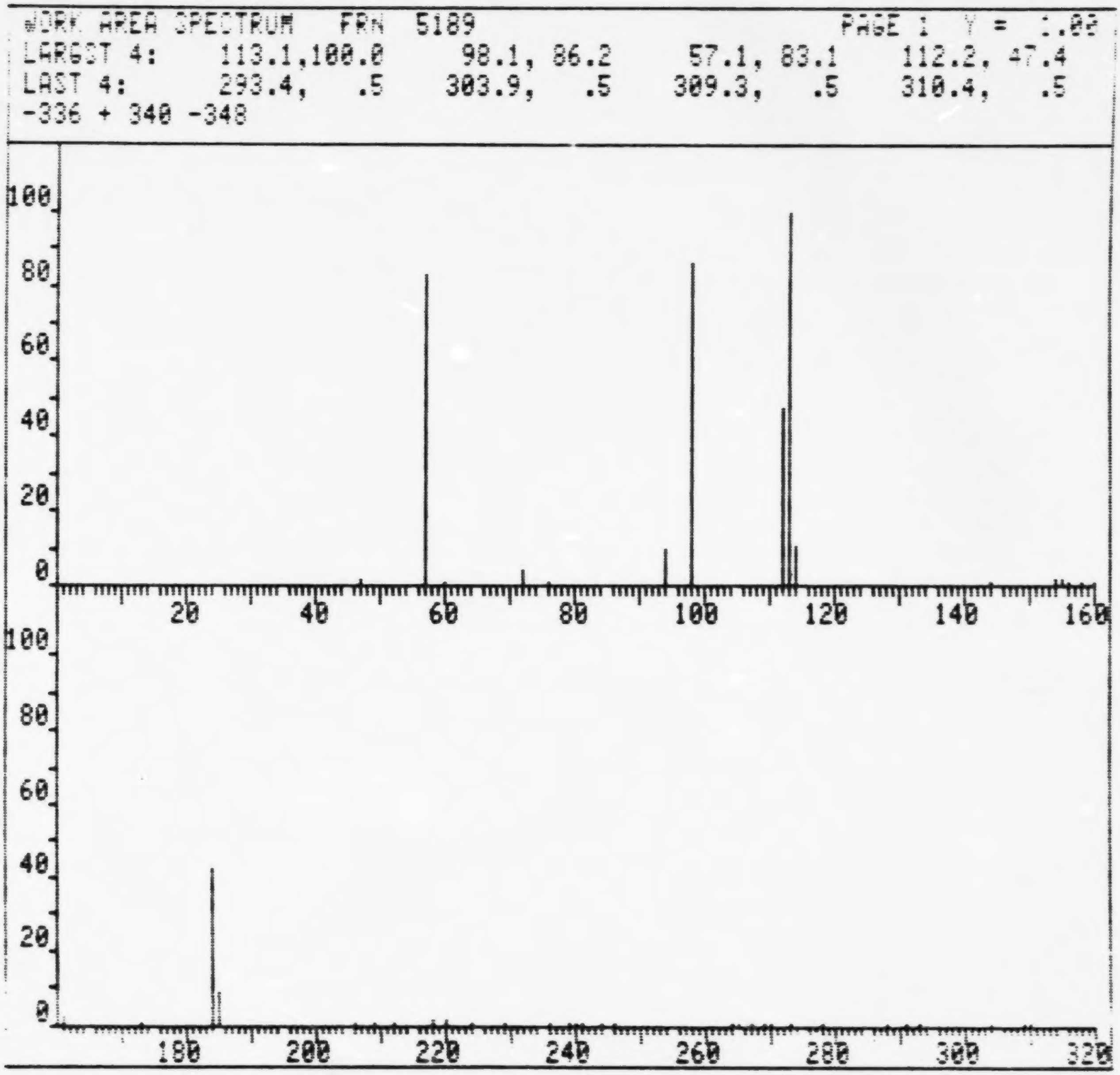
```
WORK AREA SPECTRUM    FRN  5189                                PAGE 1  Y =  1.00
LARGST 4:       98.1,100.0      57.1, 84.4      82.2, 61.4      99.1, 41.3
LAST 4:        297.1,    .2    299.1,    .3    307.0,    .3    318.5,    .3
+ 272 -265 -279
```

Figure 94. Mass Spectrum #272 in GC/MS of Volatiles from Oil #24



```
WORK AREA SPECTRUM   FRN  5189                              PAGE 1   Y =  1.00
LARGST 4:      127.0,100.0    126.2, 75.9    152.2, 62.8    153.2, 18.0
LAST 4:        280.8,  1.5    294.8,   .8    302.2,   .8    316.4,   .8
-273 + 284 -295
```

Figure 95. Mass Spectrum #284 in GC/MS of Volatiles from Oil #24



```
WORK AREA SPECTRUM   FRN  5189                    PAGE 1   Y =  1.00
LARGST 4:     113.1,100.0     98.1, 86.2     57.1, 83.1     112.2, 47.4
LAST 4:       293.4,    .5   303.9,    .5   309.3,    .5   310.4,    .5
-336 + 340 -348
```

Figure 96. Mass Spectrum #340 in GC/MS of Volatiles from Oil #24

Analyses by Direct Injection Mass Spectroscopy

As reported above, a very major problem with the analyses of these samples is their very limited volatility through the gas chromatograph/ mass spectrometer analytical system. The Hewlett-Packard 5985A system also has a sophisticated temperature-programmed direct inlet system for the mass spectrometer. Fractions 16-2, 16-3 and 12-3 were subjected to this "direct probe" analysis. The temperature programming was one minute at 50°C, then 10°C/minute to 350°C. By visual examination of the sample container, the light yellow fraction 16-2 seemed to be totally volatilized by this procedure. There were minute residues of both (dark brown) Fractions 16-3 and 16-2.

The first thing demonstrated by these analyses was that both 16-2 and 16-3 have aromatic ring-based components as their major volatiles content (see Figure 97) while 12-3 has largely aliphatic-based compounds (see Figure 98). This latter finding may already contain the reason why 12-3 is of low bio-activity. There also was demonstration of time (temperature) dependence on boil-off of materials of increasing "molecular weight" as shown by the patterns of Figures 99 through 102. (It seems strange that so much of this type of sample will be volatile under good vacuum but only out to about 150°C and this may indicate a problem with direct insertion temperature programming as presently operated by the analytical system. Probe temperature rise may be affected by source temperature.) It is not expected that the "separation by molecular weights" suggested by the data in Figures 99 through 102 can be directly interpreted by mass spectra since the sample contains literally hundreds of components. That such an interpretation is indeed impossible is shown by the data in Figure 103 whose only value is that it confirms the effect of time-temperature in increasing the average mass range in sequential spectra. A possibly important piece of data recovered from this effort is that oil Fraction 16-3 has a greater absolute abundance of "higher ion mass" - producing compounds (i.e., at mass above 224) than does either oil Fraction 16-2 or 12-3 when tested by this method. (See Figure 104.)

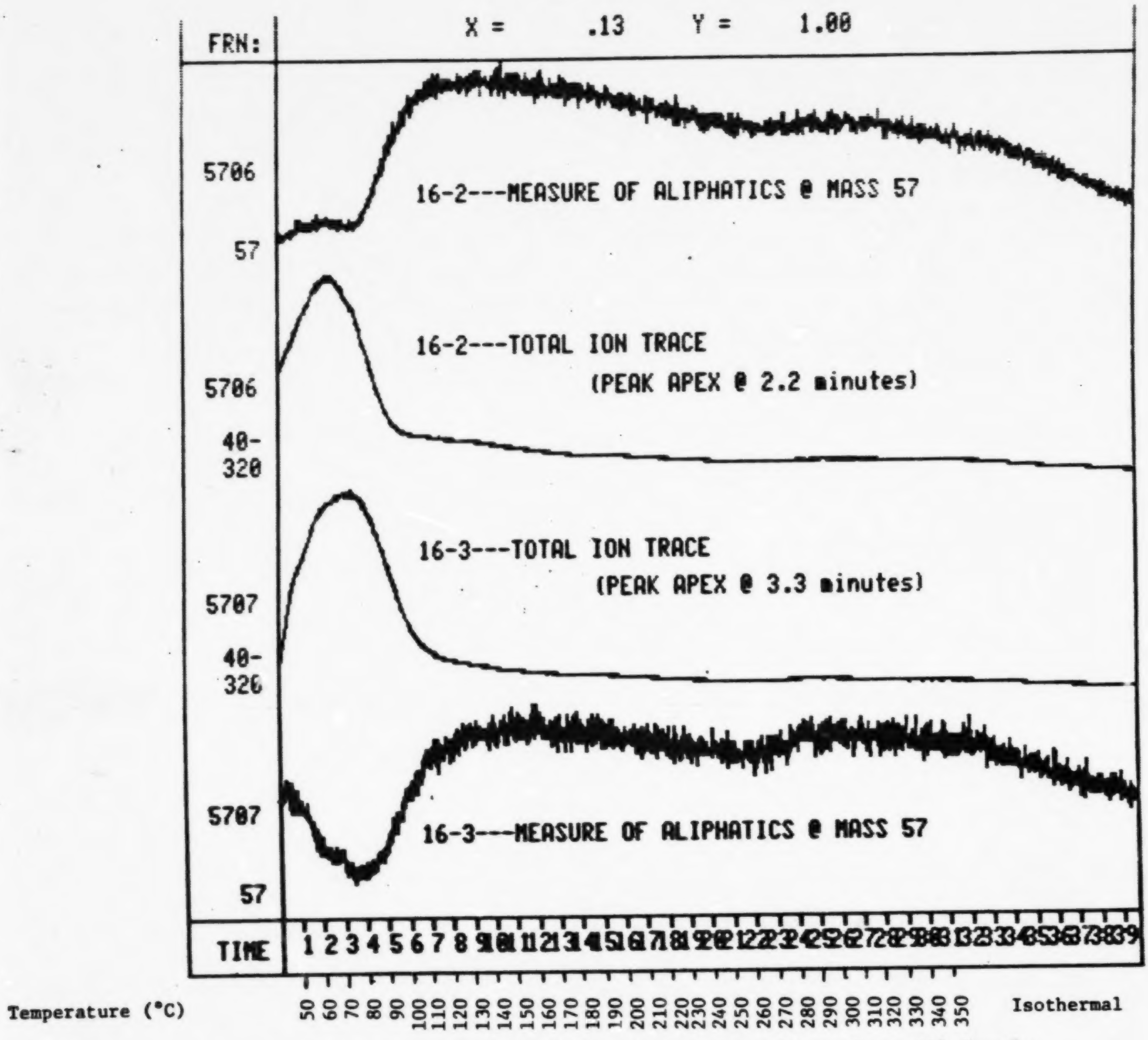


Figure 97. Measure of Aromatics/Aliphatics in Two Fractions of an Oil Sample

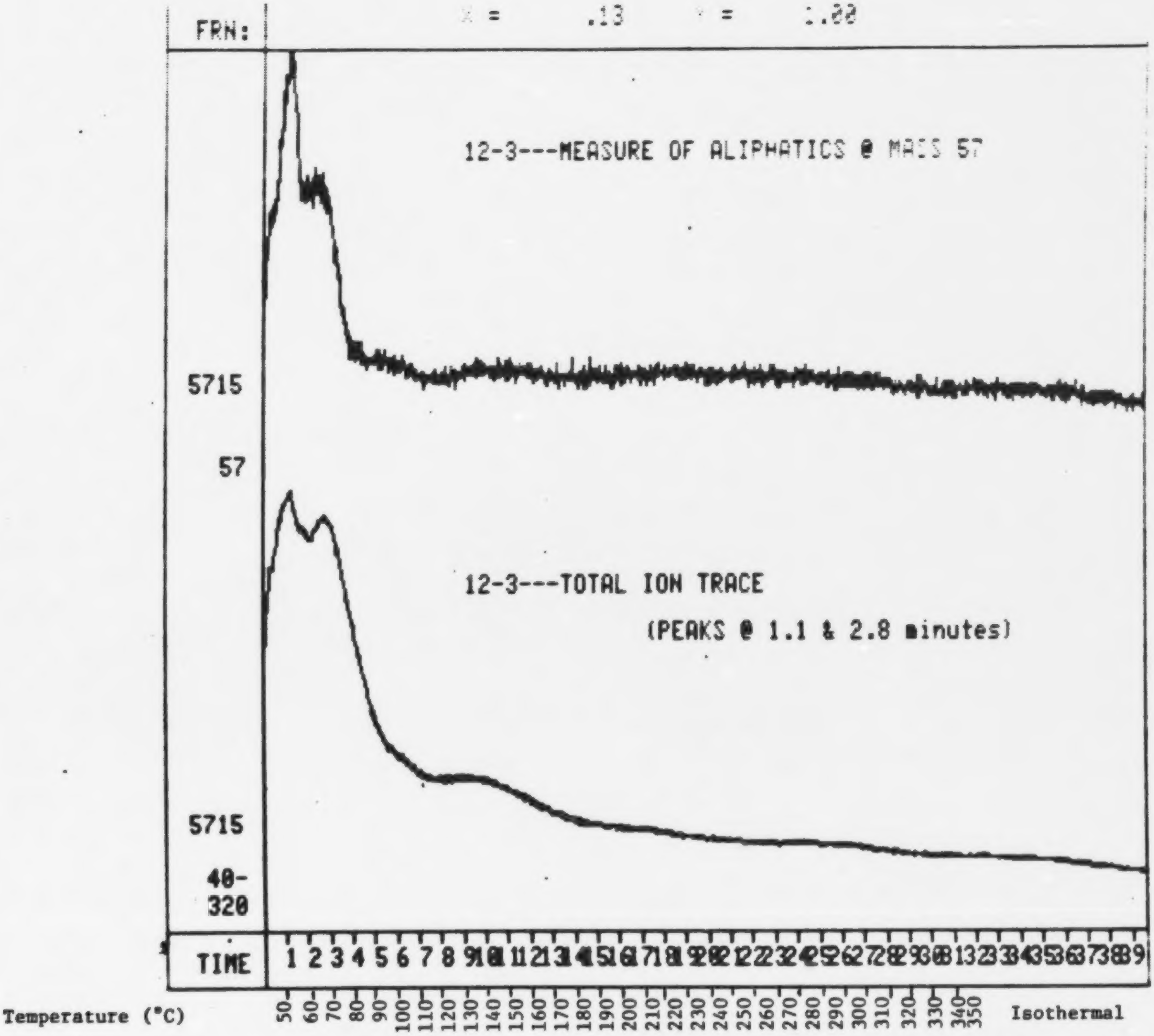
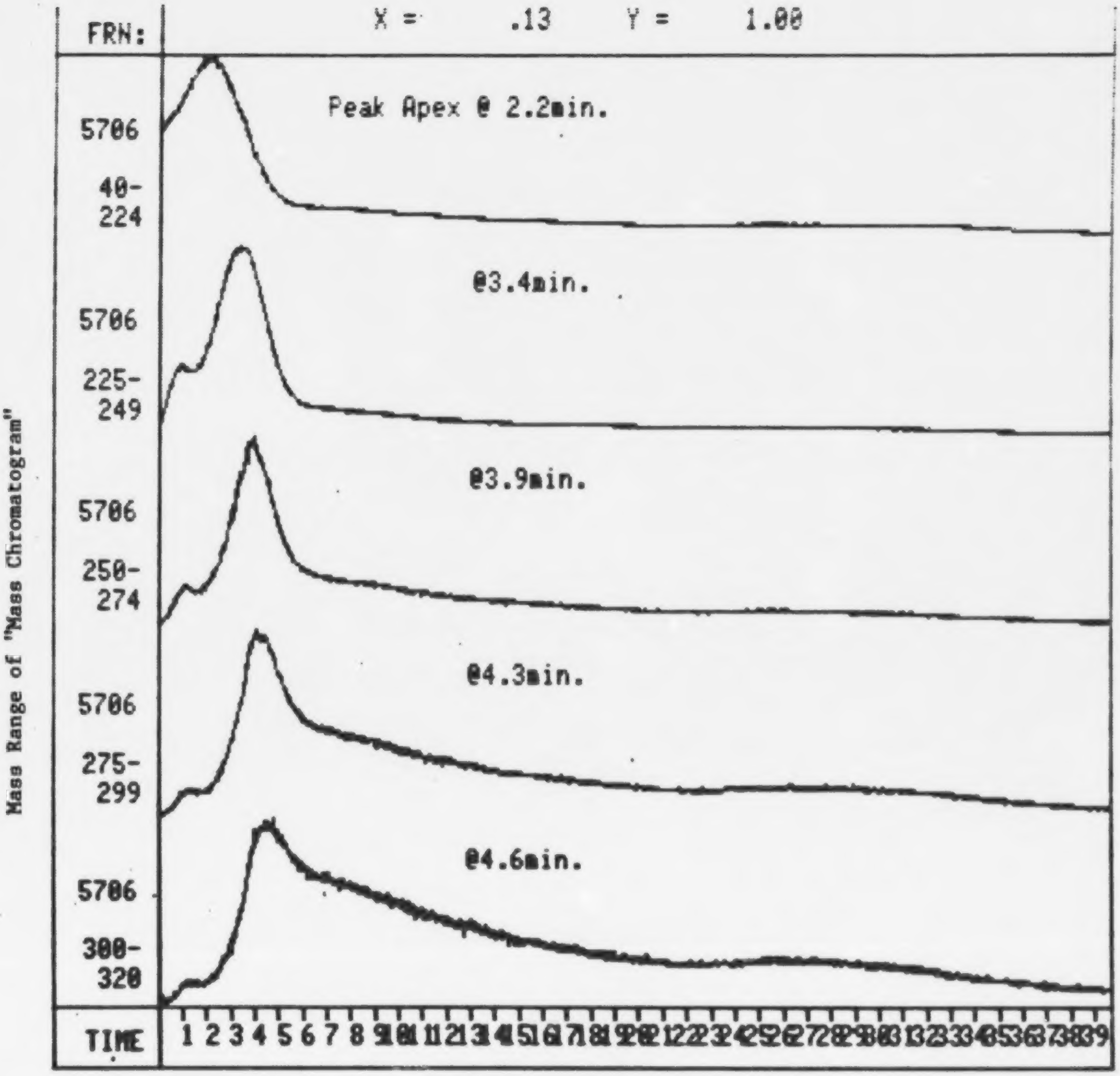


Figure 98. Measure of Aromatics/Aliphatics in a Fraction from Another Oil



Time into Analysis (Minutes)

Figure 99. Abundance of Selected Masses in Ionized, Unresolved Fraction 16-2

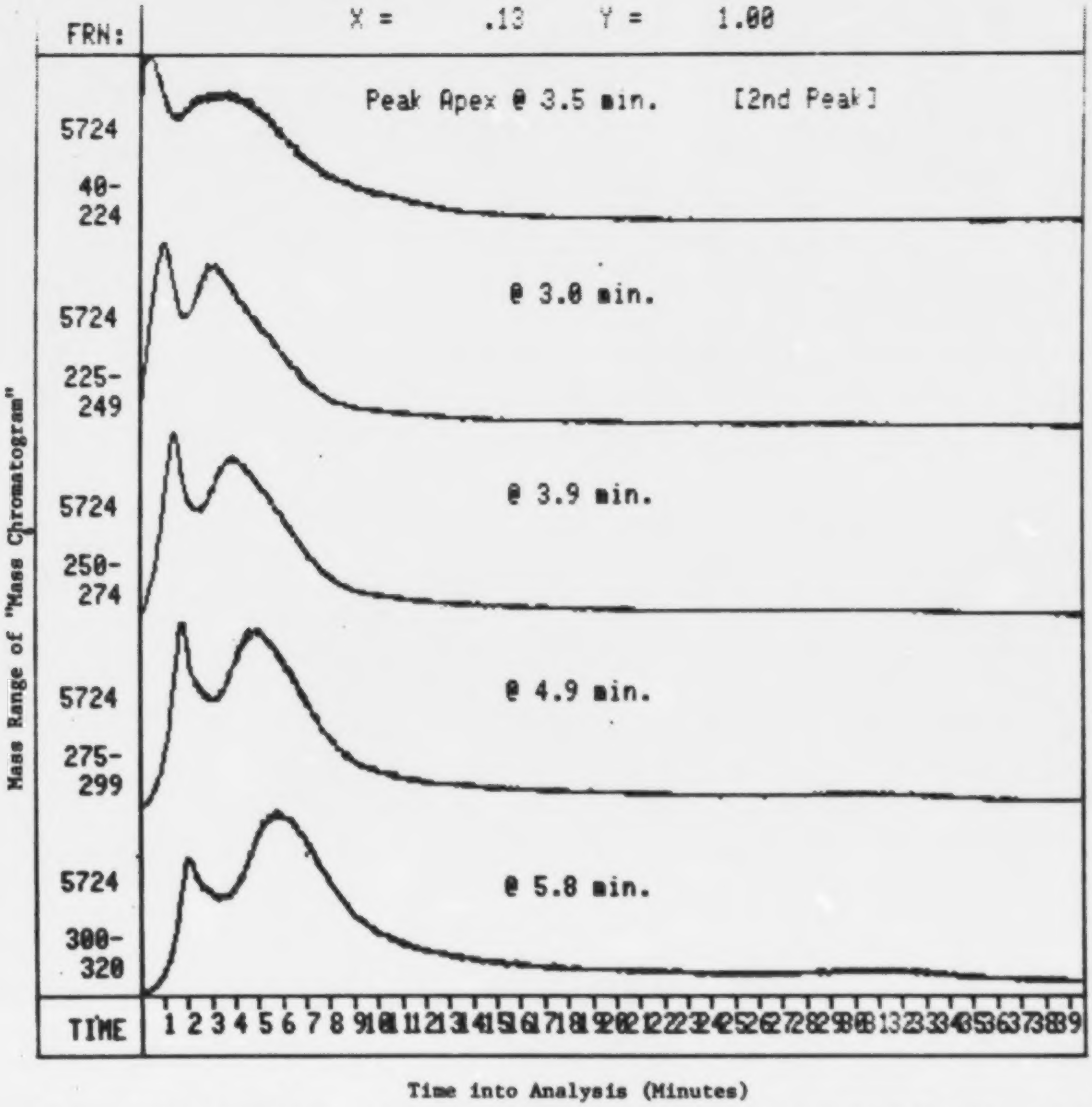
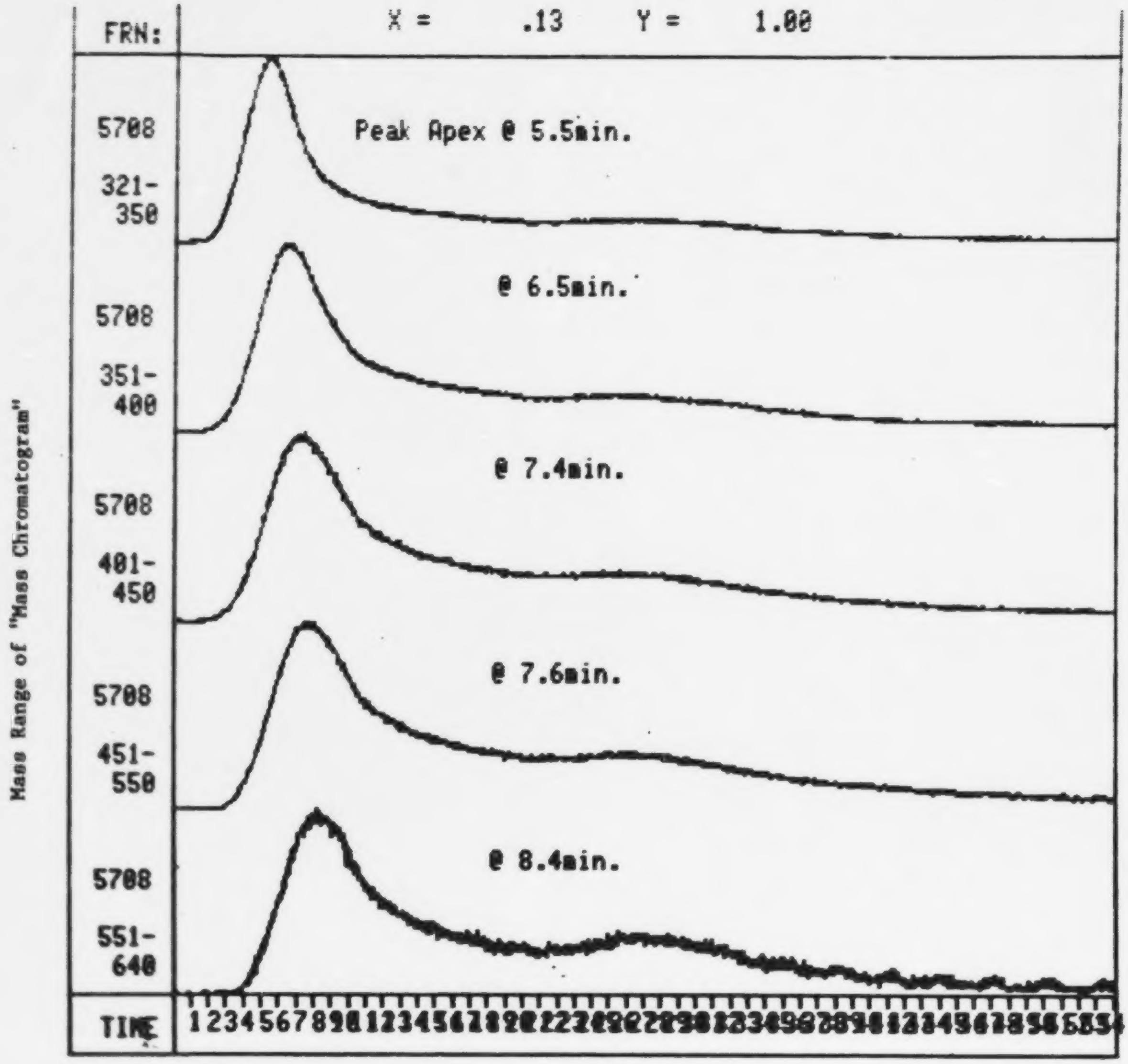


Figure 100. Abundance of Selected Masses in Ionized, Unresolved Fraction 16-3



Time into Analysis (Minutes)

Figure 101. Abundance of Selected Masses in Ionized, Unresolved Fraction 16-3 - Higher Mass Ranges

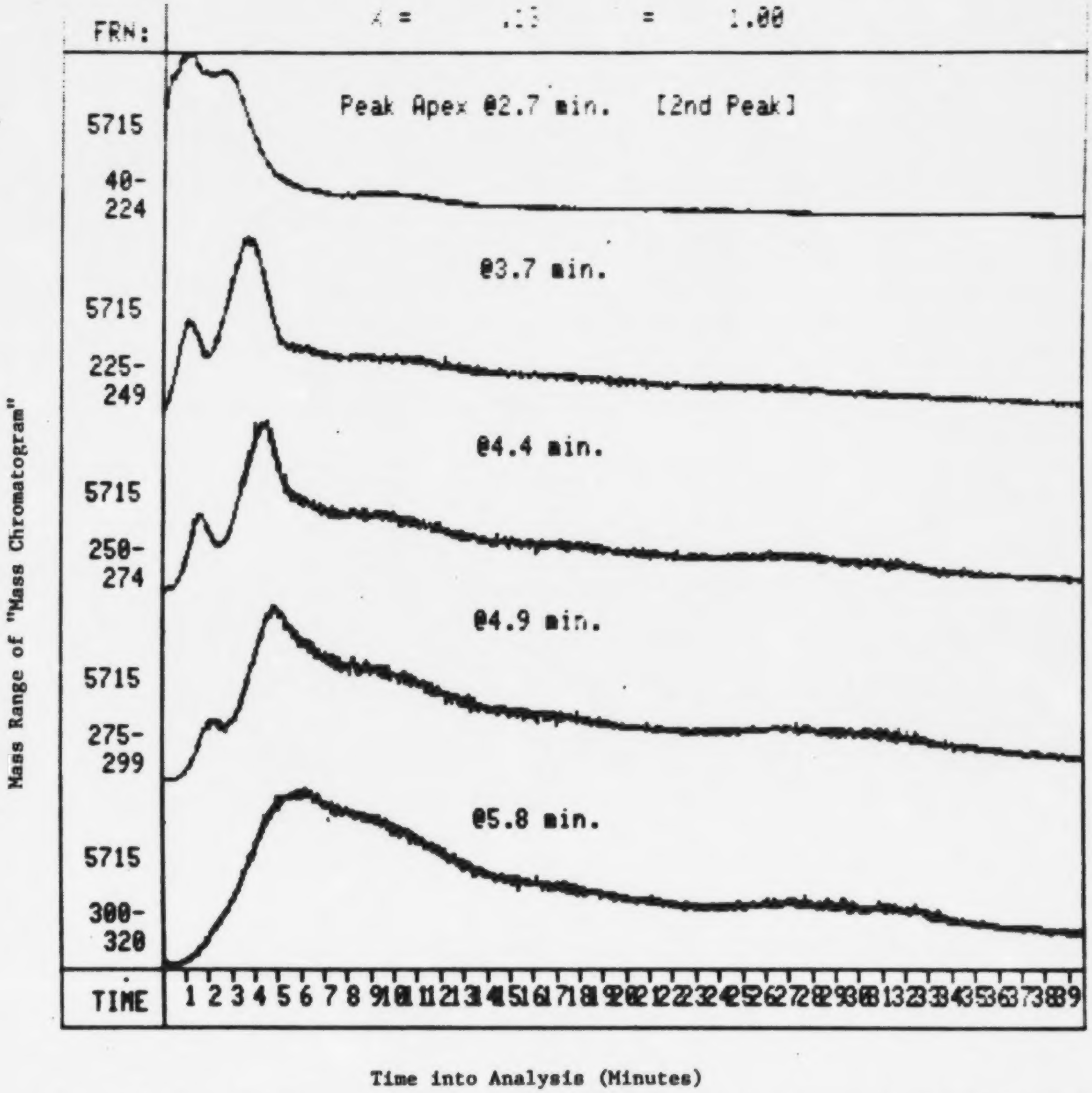
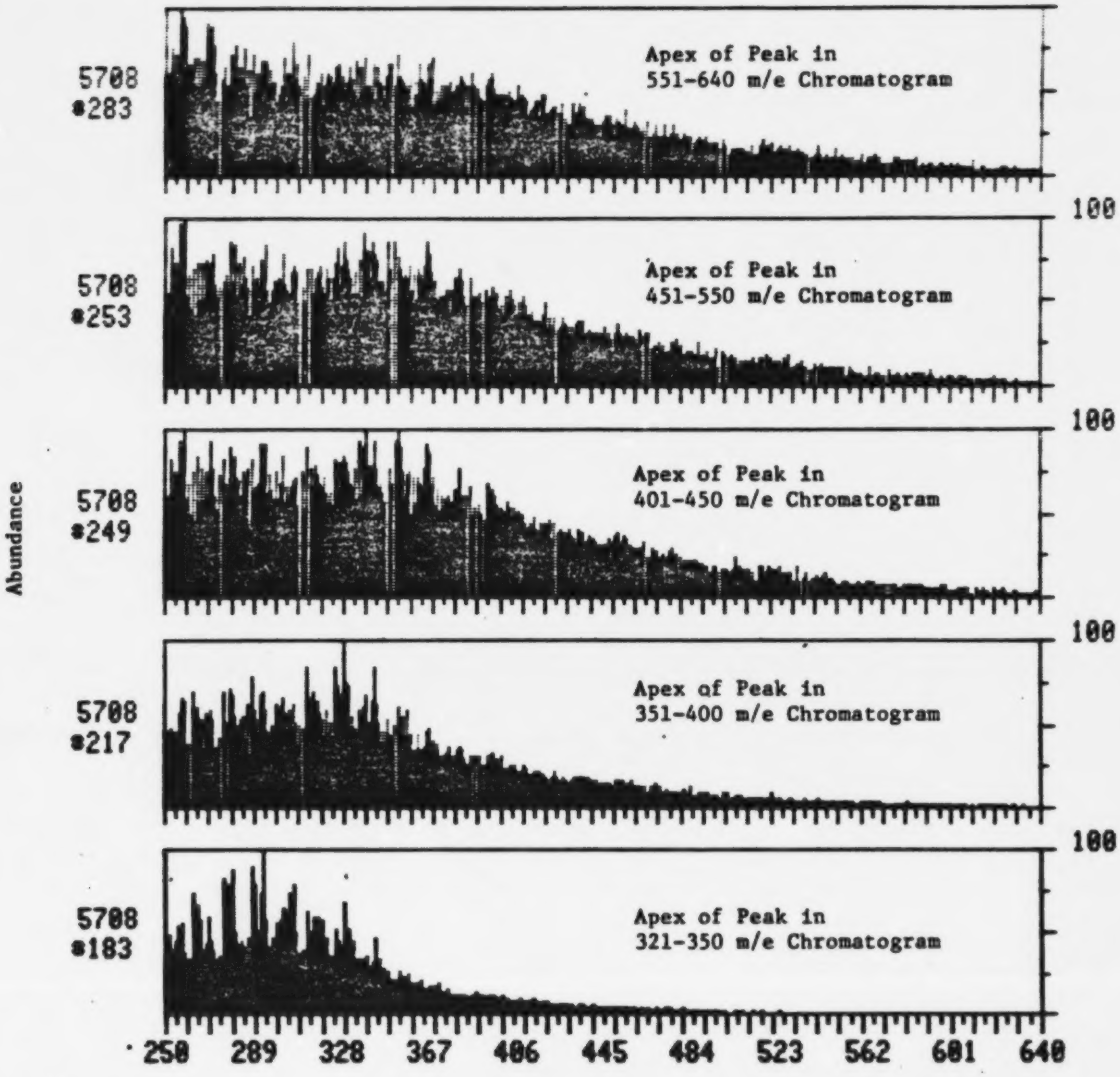


Figure 102. Abundance of Selected Masses in Ionized, Unresolved Fraction 12-3



Mass to Charge Ratio (m/e)

Figure 103. Mass Spectra in Unresolved Fraction 16-3 As Taken at the Peak Apices of Figure 5

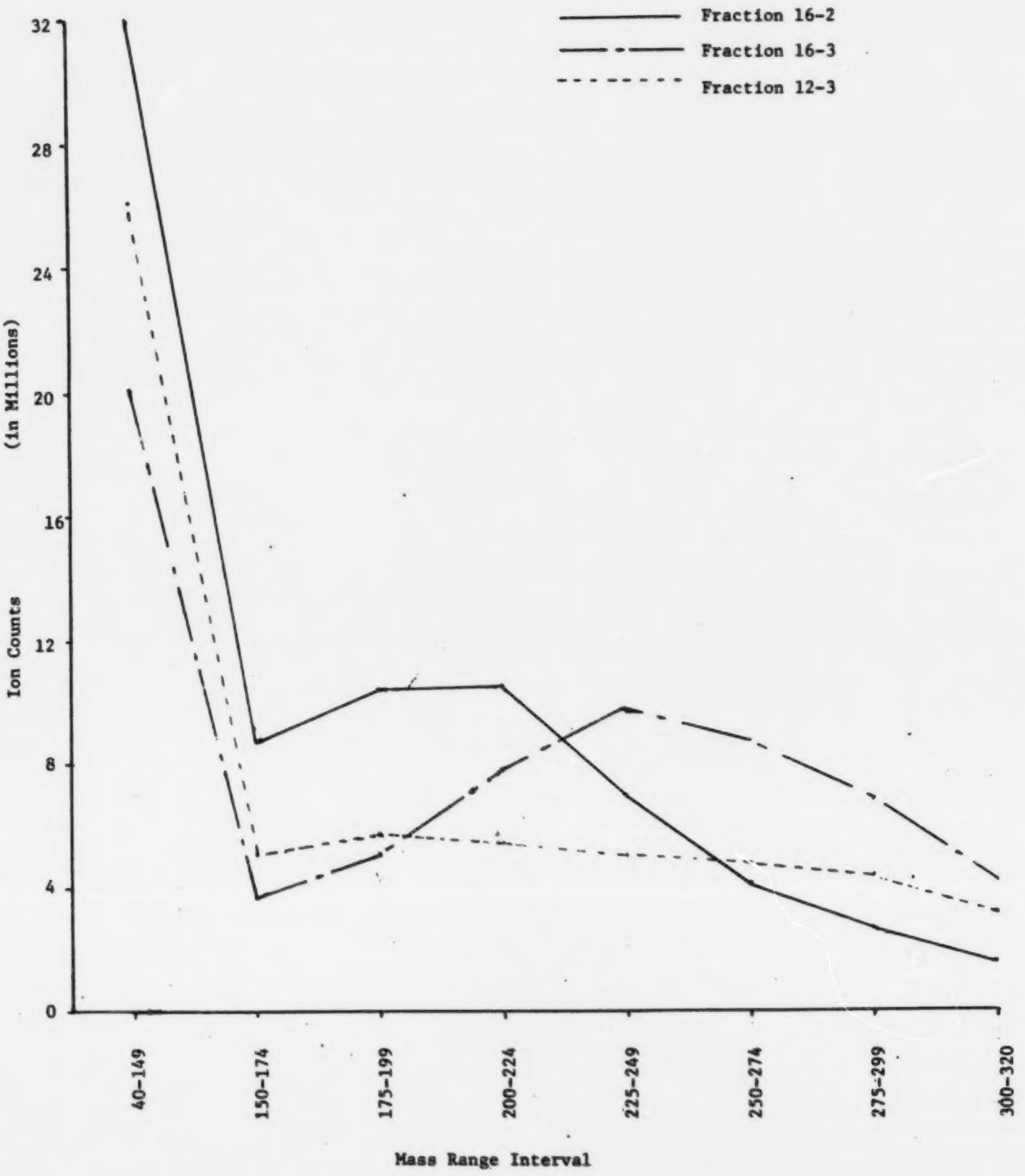


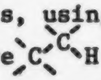
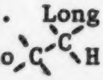
Figure 104. Mass Distribution in Ionized, Unresolved Fractions from Oil Samples

Infra-Red Examination

Infra-red examination would not be expected to be overly productive due to the massive number of compounds in a residual fuel oil. However it can reveal structural features and occurrence of functional groups in the samples. And it is one of the few techniques that can be applied to non-volatile material.

As Received Oils

The infra-red spectra of the first 36 as received oils were compared leading to the following comments:

1. The samples are not all homogeneous. We have detected minor differences in the spectra of different samplings from the same oil.
2. Differences among the oils show up primarily due to varying aromatic content (amount and type). A lesser distinction due to aliphatic content can be discerned.
3. All the oils show absorption at 1033 cm^{-1}, which may be due to S-0 bonds. This does not vary enough to "classify" the oils. Also, any interpretation based on 1033 cm^{-1} band may be misleading because of the presence of weak aromatic absorption in this region. This conclusion should therefore be regarded as highly tentative.
4. The aromatic content variation does permit a classification into four types, using the region 700-900 cm^{-1}. The 700-900 cm^{-1} region is where  bendings absorb (the C-C being part of a ring). Long chain aliphatics also show absorption at 720 cm^{-1} due to  bendings when the carbon skeleton is longer than four carbons.

Type I. A quartet (870, 810, 745, 721 cm^{-1}) with the two central bands dominant. Oils:

1
2
3
4D
6
7
10
11A^{+}
12A^{+}
13^{+}
14
15
16
17
20
21
22+
23
26^{+}
27^{+}
28+
34
35
36^{+}

Where + indicates additional bands in the 700-900 cm^{-1} region.

Type II. Same as Type I, but without (or much less) of 721 cm^{-1}.

Oils:

24
25
33

Type III. Same as Type I, but 721 cm^{-1} is the strongest band of the quartet.

Oils:

(5A)*
8
9
18
19
30
31
32

Type IV. Strong 782 cm^{-1}; distinct 1510 cm^{-1}.

Oil: 29 (only one)

5. The spectra are absorbance plots except for oils 1, 2, 3, 4D which are transmission plots and so marked. The absorbance formats are usually plotted with the 1375 cm^{-1} full-scale and with the 2922 band full-scale. Absorbance is proportional to concentration, so comparison of the oils is facilitated.

---

*5A has a split band (or doublet) centered at 725 cm^{-1} in some samplings, but the 720 cm^{-1} band alone in other samplings. The variation is due to non-homogeneity.

(Allowances must be made for baseline and band shape variations, but quantitative eyeball comparisons can be made.) Some tentative conclusions:

a. Oil 25 has the highest aromatic content. Other "highs" are oils 10, 24, 29, and 33. "Lows" are oils 8, 30, and 32. The others might be called "in-betweens."

b. Oil 29 is unique. It probably contains a relatively-volatile aromatic.

c. Oil 25 has the strongest 1033 cm^{-1} band. Therefore, it may have the most S-O bonds. However, note the caution given in Item 3, above.

The infra-red spectra of all 36 oils are appended as Appendix G.

Column Pre-Separation Fractions

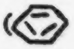
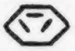
In an attempt to see if more information could be gathered on the unresolved volatiles/non-volatiles in these fractions, an infra-red examination was performed. Although it was found impossible to identify individual components the study was important in that it does show the destination of compounds with sulfur-oxygen linkages. The IR analysis report follows:

Fraction I; Hexane Solvent

This fraction consists predominantly of crystalline aliphatic hydrocarbons. The extent of the amorphous character is given by the intensity of the 1307 cm^{-1} band. Weak bands between 1000-870 cm^{-1} indicate the type and amount of unsaturation and those between 1225-1075 cm^{-1} indicate the type of C-C linkages.

Fraction II; Hexane with 15% Toluene

Fraction II is characterized by the largest amounts of aromatic hydrocarbons which normally would not elute with non-aromatic hydrocarbons.

To determine the major constituents in these fractions, the first 10 oils and oils 24, 25 and 29 were examined on a computer controlled Digilab Fourier Transform Infrared Spectrometer. A wide range of aromatics characterized by their respective sharp peaks were observed. Also observed were complex unresolvable bands at 875, 810 and 744 cm^{-1}. These bands were so strong that the only features worth spectral compensations were the peaks outside the 880-740 cm^{-1} range. Dibenzyl ( -CH$_2$-CH$_2$-  mol. wt. 182) was the only compound identified unambigously because of its relative large proportion and infrared peaks well isolated from the above interferences.

Identification of individual major components is, therefore, not possible by straight or digitally-compensated infrared spectra. Paper chromatography followed by infrared or LC/IR may provide unambiguous identification on chromatographically resolvable peaks.

Fraction III; Methylene Chloride

This fraction is characterized by compounds containing sulfur and acid carbonyl groups, probably attached to both aromatic and aliphatic chains. $R\text{-}\underset{\underset{O}{\Downarrow}}{S}\text{-}R'$ were observed to be the predominant, or maybe the only, moiety in which sulfur and oxygen atoms may have been linked.

Fraction IV; Ether + 3% Ethanol

This fraction contains the largest amount of sulfur-oxygen linkages. Also present are acid carbonyl (1700 cm^{-1}), aromatics (1600 cm^{-1}) and olefin groups (1650 cm^{-1}).

Fraction V; Methanol

The infrared spectrum of this fraction is very similar to the previous fraction except for an additional strong component at 1140 cm^{-1}. It is also observed to elute from a blank column and is, therefore, identified as Na_2SO_4. Small amounts of carbonates (1450, 879 cm^{-1}) are also washed down by methanol.

Fraction VI; Methylene Chloride + 3% Ethanol

The organic constituents are very similar to those obtained in the above fraction. Much more carbonates leached out than in Fraction V.

Fraction VII; Dioxane + 3% Ethanol

Of the last four fractions, this fraction had the lowest amount of S-O linkages. This fraction also contained ester type groups, probably attached to aromatics. Surprisingly, nothing leached out from the blank column.

Ten Sub-Fractions from an Oil Fraction #3

As explained under the bioassay discussed above, a Fraction #3 from oil #16 was obtained as 10 sub-fractions in an attempt to further isolate mutagenic factors. Infrared examination of the 10 fractions showed there was some degree of chemical separation affected among the fractions. The infrared spectra were recorded by dissolving the sample in CS_2, casting a thin film on 8 mm diameter KBr plate and placing it in the sample chamber of the spectrometer. Digilab's Fourier Transform Spectrometer (FTS-14) was used to record the spectra between 3800-500 cm^{-1}. Comparison of the corresponding cuts shows different amounts of aromatics, sulfur, acid and unsaturations. Also the types of aromatics differ. The earlier cuts resemble Fraction 3 while the later cuts resemble Fraction 4 of the same oil; cut 7 being a tentative dividing line. As more methylene chloride is passed through the column, polar groups such as carbonyl, longer chain aliphatics (or less branched aliphatics) start eluting. For the first seven cuts, the amount of aromatics (given by the relative peak height of 1605 cm^{-1} band) stays the same but the type and amount of substitutions (given by 900-700 cm^{-1} bands) varies. After the seventh cut the amounts and types of aromatics shows a different trend and are accompanied by unsaturations. The amount of sulfur decreases gradually in going from 1 through 10. It must be emphasized that interpretations from infrared spectra as outlined above are based on our existing knowledge of group frequencies. These are, in general, useful for comparisons and major categorizations. Finer details without individual component separations of these multicomponent blends are not very meaningful.

DMSO Partitioning of an Oil

As seen by IR analyses, the dimethylsulfoxide partitioning procedure is quite effective in separating aliphatics from aromatics and non-sulfur from sulfur-containing moieties. The fractions obtained by this procedure do not exhibit a 1:1 correlation with our earlier column pre-separation procedure but do exhibit some common characteristics. [Fractions are keyed to previous Figure 62.]

DMSO Insolubles: Fractions G and F

Fraction G has the lowest amount of aromatics while Fraction F has the next lowest. On the overall, DMSO provides a separation between aliphatics and aromatics. These fractions also have small amounts of unsaturations and acid carbonyl functionalities.

DMSO Soluble, Hexane Soluble: Fraction B

This fraction has the highest amount of aromatics. It resembles 16-3 of column pre-separation in aromatic character. It also has small amounts of unsaturations and acid carbonyl groups.

DMSO Soluble, Hexane Insoluble (I): Fraction C

This fraction is highest in sulfur, unsaturations and acid carbonyl groups. The amount of aromatics is moderate.

DMSO Soluble, Hexane Insoluble (II) and Toluene Solubles: Fractions D and E

These two lie somewhere between B and C in infrared absorption characteristics.

Column Pre-Separation Fractions of DMSO Partitioned Sample

The infra-red examination effort on these fractions is similar in format to that used with fractions of as received oils, as reported above. As before, present infra-red interpretation is based on:

1. Aromatic hydrocarbons (as measured by IR absorptions at 3040 cm^{-1} and 900-700 cm^{-1}).
2. Acid carbonyl functionalities (as given by the 1700 cm^{-1} band).
3. S-O linkages (as judged by the intensity of the 1033 cm^{-1}).
4. The total unsaturation (in terms of the 1650 cm^{-1} band).

Infra-red examination of the seven pre-separation fractions from the DMSO solubles of oil #16:

Fraction 1 (Hexane as eluant).

Unlike the hexane fraction of an as received sample, this fraction contains a major portion of aromatic hydrocarbons. It relates better to the hexane + 10% toluene eluate of an as received oil sample. This correlates with GC/MS analyses of these fractions. As reported earlier the general polarity of the sample onto the pre-separation column appears to affect the polarity of eluants.

Fraction 2 (Hexane + 10% Toluene as eluant).

Like the hexane cut, it is characterized by the absence of polar functional groups. The only difference from Fraction 1, above, being the absence of some sharp peaks due to their incorporation into the hexane eluant.

Fraction 3 (Methylene chloride as eluant).

It is characterized by a slight decrease in the relative proportion of the aromatic hydrocarbons and the emergence of some acid functionalities. A sharp peak at 3430 cm^{-1} is attributed to compounds with primary or secondary amine types of groupings. This would be consistent with the GC/MS findings of carbazole and its homologues in this fraction.

Fraction 4 (Ether +3% Ethanol as eluant).

This fraction contains the largest amount of acid carbonyl groups. The relative proportion of the aromatic hydrocarbons decreases and the sulfur containing compounds are about the same as in the first three fractions. Strong IR bands at 1653, 1603, 1580, 840 and 780 cm^{-1} are assigned to benzanthrone units which would be consistent with GC/MS findings.

Fraction 5 (Methanol as eluant).

This solvent elutes the largest proportion of sulfur-oxygen containing groups of any sample as examined by infra-red under this contract. In fact the IR band at 1033 cm^{-1} is stronger than the C-H deformations. DMSO is not represented in the spectra unless frequency shifts are involved.

Fraction 6 (Methylene Chloride + 3% Ethanol as eluant).

The proportion of sulfur containing compounds is much larger than the first four cuts and of course smaller than in fraction 5.

Fraction 7 (Dioxane + 3% Ethanol as eluant).

This elution shows a further decrease in sulfur and a few sharp peaks due to a family of aromatic compounds that were not resolved by the previous solvents. The unidentified species absorbs at 1180, 730 and 695 cm^{-1}.

Infra-red examination of the seven pre-separation fractions from the DMSO insolubles of oil #16:

Intercomparisons of the spectra from different eluants indicated that;

1. None of these fractions had sulfur, aromatic hydrocarbons or unsaturations comparable to those of DMSO solubles.
2. The "trends" are similar to those of the DMSO solubles.

3. Fraction three has a slightly higher percentage of the aromatic hydrocarbons among the seven fractions obtained from DMSO insolubles of oil #16.

4. For all practical purposes, the last four eluants gave very similar spectra except for the additional presence of Na_2SO_4 (from our column) in Fraction 5.

To summarize the infra-red effort:

1. These fractions from DMSO solubles and insolubles do not correlate on a one-to-one basis with our earlier fractions from as received oils.

2. The DMSO solubles may be thought of as consisting of aromatic hydrocarbons (the first three cuts), high acid carbonyl functionality (Fraction 4), a predominantly sulfur-containing fraction (5) and a little bit of everything in the last two fractions.

3. Most aliphatics are recovered into the DMSO insoluble fraction. Further classification of the DMSO insoluble fractions didn't prove as clearcut as with their DMSO soluble counterparts.

INORGANIC ANALYSES

Metals Content Determination

Optical Emission Spectroscopy

Twenty-five residual oil samples have been examined by optical emission spectroscopy (OES) and the results are tabulated in Table 37. Twenty-eight elements were measured with an accuracy objective of ±25% for these elements at 10 ppm and a range of 1/2 to 2X for analyses at the 1 ppm level. An appraisal has been made using residual oil reference standard SRM 1634. Table 38 tabulates the current data versus other reported estimates including the certified values.

Table 37

OPTICAL EMISSION SPECTROSCOPY RESULTS ON RESIDUAL FUEL OILS

W Sample No.	N	Ag	As	B	Ba	Bc	Bi	Ca	Cd	Co	Cr	Cu	Fe	Ge	Mg	Mn	Mo	Ni	P	Pb	Sb	Si	Sn	Sr	Ti	V	Zn	Zr	Re
1	10	<0.1	<1	<1	<0.3	<0.1	<0.1	3	<0.6	0.1	0.2	<0.1	15	<0.1	1	0.14	<0.1	14	<3	1.2	<0.3	>10	<0.1	<0.3	0.1	53	0.25	<0.1	D
2	3	<0.1	<1	0.3	<0.3	<0.1	<0.1	10	<0.1	0.4	0.2	<1	9	<0.1	10	<0.1	0.6	22	<3	0.75	<0.3	10	3	<0.3	0.1	122	0.7	<0.1	D
3	3	<0.1	<1	<0.5	<0.3	<0.1	<0.1	3	<0.1	0.1	0.3	<1	17	<0.1	2	<0.1	0.15	15	<3	0.25	<0.3	>10	2	<0.3	0.1	70	0.8	<0.1	D
4	10	<0.1	<1	1	<0.3	<0.1	<0.1		<0.1	0.1	0.2	<1	10	<0.1	>10	0.2	0.08	37	<3	0.1	<0.3	>10	<0.1	<0.3	0.1	209	0.3	<0.1	ND
5	16	<0.1	<1	0.1	<0.3	<0.1	<0.1	2	<0.1	1	0.2	<1	12	<0.1	0.3	<0.1	<0.1	23	<3	<0.1	<0.3	>10	<0.1	<0.3	2	11	0.35	<0.1	ND
6	0.3	<0.1	<1	0.1	<1	<0.1	<0.1	0.2	<0.3	<0.1	<0.1	<1	<2	<0.1	0.1	<0.1	< 2	3	<3	<0.1	<0.2	10	<0.1	<0.3	<0.1	11	<0.3	<0.1	ND
7	10	<0.1	<1	0.1	<1	<0.1	<0.1	0.5	<0.3	0.3	0.3	<1	17	<0.1	3	0.2	<0.1	26	<3	0.6	<0.2	>10	<0.1	<0.3	0.1	90	2	<0.1	D
8	1	<0.1	<1	0.5	<1	<0.1	<0.1	0.8	<0.3	0.1	0.1	<1	2	<0.1	0.1	<0.1	0.1	11	<3	<0.1	<0.2		<0.1	<0.3	0.2	33	<0.3	<0.1	D
9	3	<0.1	<1	5	1	<0.1	<0.1	2	<0.3	1	0.1	<1	13	<0.1	1	<0.1	0.5	23	<3	0.1	<0.2		<0.1	<0.3	0.1	14	1	<0.1	D
10	130	<0.1	<1	5	<1	<0.1	<0.1	2	<0.3	1	0.3	<1	21	<0.1	1	0.2	1.2	19	<3	0.8	<0.2	>10	<0.1	<0.3	5	36	0.4	<0.1	D
11	4	<0.1	<1	10	1	<0.1	<0.1	5	<0.3	1	0.2	<1	13	<0.1	1	0.2	0.2	17	<3	<0.3	<0.2	>10	<0.1	<0.3	0.2	26	0.5	<0.1	D
12	5	<0.1	<1	10	1	<0.1	<0.1	5	<0.3	1	0.2	<1	19	<0.1	1	0.3	0.25	27	<3	<0.3	<0.2	>10	<0.1	<0.3	0.2	43	0.6	<0.1	D
13	3	<0.1	<1	1	<1	<0.1	<0.1	2	<0.3	0.1	0.2	<1	12	<0.1	0.3	0.15	<0.1	17	<3	0.8	<0.2	3	<0.1	<0.3	0.1	29	0.3	<0.1	D
14	7	<0.1	<1	5	<1	<0.1	<0.1	3	<0.3	0.1	0.2	<1	26	<0.1	0.5	0.15	<0.1	18	<3	1.2	<0.2	>10	<0.1	<0.3	0.1	27	0.4	<0.1	D
15	5	<0.1	<1	0.3	<1	<0.1	<0.1	0.2	<0.3	0.2	0.2	<1	21	<0.1	0.5	0.15	<0.1	30	<3	0.8	<0.2	10	<0.1	<0.3	0.2	56	0.4	<0.1	ND
16	87	<0.1	<1	0.2	<0.3	<0.1	<0.1	2	<0.1	0.3	0.2	<1	13	<0.1	3	0.5	0.14	36	<3	1.4	<0.3	>10	<0.1	<0.3	2	60	0.4	<0.1	ND
17	20	<0.1	<1	2	<1	<0.1	<0.1		<0.1	<0.1	0.1	<1	12	<0.1	3	0.2	<0.1	10	<3	0.3	<0.3	>10	<0.1	1	1	7	1	<0.1	ND
18	3	<0.1	<1	1	2	<0.1	<0.1	2	<0.1	0.3	0.2	<1	36	<1	0.2	0.2	<0.1	12	<3	0.3	<0.3		<0.1	<0.3	0.1	13	1	<0.1	ND
19	1	<0.1	<1	0.3	<1	<0.1	<0.1	<1	<0.1	<0.1	<0.1	<1	6	<0.1	0.1	<0.1	<0.1	8	<3	<0.1	<0.3		<0.1	<0.3	<0.1	20	<1	<0.1	ND
20	21	<0.1	<1	2	<1	<0.1	<0.1	10	<0.1	0.3	0.3	<1	24	<0.1	3	0.2	0.8	21	<3	0.5	<0.3	>10	<0.1	<0.3	2	74	1	<0.1	ND
21	26	<0.1	<1	1	<1	<0.1	<0.1		<0.1	<0.1	2	<1	28	<0.1	3	1	0.4	10	<3	0.5	<0.3	>10	<0.1	<0.3	2	21	2	<0.1	ND
22	45	<0.1	<1	2	1	<0.1	<0.1	10	<0.1	1	0.2			<0.1	2	0.2	0.5	17	<3	0.2	<0.3	>10	<0.1	<0.3	2	20	1	<0.1	ND
23	33												18					61								39			
24	42	<0.1	<1	2	<1	<0.1	<0.1		<0.1	<0.1	0.5	<1	22	<0.1	10	0.3	0.8	52	<3	0.8	<0.3	>10	<0.1	<0.3	3	34	2	<0.1	ND
25	43	<0.1	<1	1	<1	<0.1	<0.1	3	<0.1	<0.1	0.3	<1	43	<0.1	2	0.2	0.8	1	<3	0.3	<0.3	>10	<0.1	<0.3	3	0.3	<1	<0.1	ND
$\bar{X}$													17.1					21.2								44.7			

D - Detected
ND - Not detected

An analysis of the table confirms that accuracy objectives to this point are being met.

Two observations concerning accuracy can be extracted from Table 38. The first relates to V for which a mean value of 271 ppm has been obtained by OES. This compares favorably with the 266 ppm estimate of NASA; however, it is negatively biased by 15% when compared with the certified 320 ± 15 ppm level. It is suspected that the OES measurement is low and this may be associated with an apparent slight difference in bulk density between standards and sample. A better estimate of any bias comes when atomic absorption values for V, Ni, and Fe on all samples are compared against OES analyses.

The second observation would have to deal with accuracy at the 1 ppm region. As can be noted from Table 38, there are a limited number of elements for comparisons in this region. What evidence that is available supports a ±50% accuracy range well within the objective sought for the method. For elements for which no comparisons can be made, measurements of precision through replication will have to serve as a basis for estimating accuracy.

Assessing the results for the 25 fuel oils examined, the initial conclusion is that the oil is essentially free of metal contamination. The elements that are not included in this analysis are the alkalies and it is not anticipated that they will cause gross effects. The mean values for the elements usually associated with crude oil, V and Ni with the addition of Fe as a contaminant though handling is low. The values obtained are:

$\overline{X}$	ppm
V	45
Ni	21
Fe	17

Table 38

COMPARISON OF CURRENT VALUES ON RESIDUAL OIL STANDARD SRM 1634 WITH OTHER REPORTED LEVELS

Values in ppm

			NASA[1]		
Element	Current Replication	Earlier Work		Standard Dev. %	Certified Result
V	267	263/282	266	7	320 ± 15
Ni	27	36	39.5	6	36 ± 4
Fe	12	13.5/17	12.4	13	13.5 ± 1
Zn	0.15	<5	0.48	25	0.23 ± 0.05
Cr	0.1	0.3	0.116	30	
Mn		0.15	0.19	—	(0.12)[2]
Mo	0.22	(1)			
Co	0.2	<0.3	0.25	4	

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[1] Trace Elements in Fuel, Suresh P. Babu, Ed., Advances in Chemistry Series - 144, American Chemical Society, Washington, D.C. 91975) p. 116.

[2] Uncertified.

They ranged from 0.3 to 209 ppm for the V and 1 to 61 for Ni. Since V and V and Ni are more generally associated with the asphaltic fraction of crude oil, one may infer that their absence in No. 6 sample might indicate a higher lighter oil content. One sample, No. 25 in Table 37, was unique in its near absence of V and Ni and should have properties differing from sample No. 4 with its 209 ppm V content.

Other metals that are of environmental concern, Pb, Cr, Mn, Mo, Co, Cd, etc., if present, are at the ≦1 ppm concentration.

One element, Rhenium, for which no calibration has been made, has been noted when detected. It is not expected to exceed 1 ppm in the samples in which it is present. Along with other characteristics it may help to identify the source of oil when identification is uncertain.

Some of the sample fractions from dioxane and toluene partitioning of residual fuel oils were subjected to optical emission spectroscopy. The data on the partitioning work is given in Table 39:

Table 39

EXTRACTION OF AS RECEIVED SAMPLES WITH DIOXANE AND TOLUENE

W Sample Number	Percent Dioxane Soluble	Percent Dioxane Insoluble, Toluene Soluble	Percent Toluene Insoluble
2	92.7	7.2	0.12
7	96.5	3.44	0.04
	96.1	3.72	0.19
10	96.4	3.51	0.13
16	94.8	5.14	0.07
NBS-1364	91.2	8.82	0.02
	91.5	8.51	0.03

The optical emission spectroscopy results as available are shown in Table 40. [Note: analysis was for vanadium, iron and nickel only.] It does appear that partitioning such as with dioxane and toluene provides a means of greatly concentrating metallo-organics into a discrete fraction. This would have to be checked with many more samples.

Atomic Absorption Spectroscopy

The elements that have a more common geological identification with petroleum; vanadium, nickel, and iron were determined by atomic absorption spectroscopy. Because the methodology and the instrumentation combine simplicity with a high level of accuracy it has gained wide acceptance as an analytical chemistry tool. The analytical results that were obtained on the first 25 residual fuel oils are listed in Table 41.

Table 40

METALS CONTENTS OF DIOXANE/TOLUENE FRACTIONS OF AS RECEIVED SAMPLES

		Metals Contents in Parts-per-Million				Metals Contents in Microorganics (Materials Balance)	
W Sample Number	Metal	As Received Sample	Dioxane Soluble	Dioxane Insoluble Toluene Soluble	Toluene Insoluble	As Received Oil	Σ(Dioxane Sol.; Dioxane Insol-Toluene Sol.; Toluene Insol.)
2	Vanadium	113	60	900	3500	113	124
	Iron	20	8	1000	6000	20	22
	Nickel	27	20	200	1100	27	37
7	Vanadium	84	--	2680*	448	84	--
	Iron	14	--	184*	3960	14	--
	Nickel	28	--	804*	208	28	--
10	Vanadium	45	--	1210*	277	45	--
	Iron	5	--	145*	5077	5	--
	Nickel	8	--	210*	215	8	--
16	Vanadium	70	--	1340*	250	70	--
	Iron	13	--	142*	7778	13	--
	Nickel	34	--	605*	61	34	--
NBS-1364	Vanadium	267	--	1620 900	N.A.	--	--
	Iron	13.5	--	160 118	3583 5875	--	--
	Nickel	32	--	194 140	N.A.	--	--

*These values were calculated from the difference between the known values for each element in the as received sample and that fraction recovered in the toluene insoluble. It was assumed that the dioxane soluble was neglible. The dioxane insoluble, toluene soluble fractions were unavailable for analysis.

N.A. The fraction of sample recovered, ∿1 mg, was too small for analysis.

Table 41

RESIDUAL FUEL OIL

V, Ni and Fe BY ATOMIC ABSORPTION SPECTROSCOPY

Westinghouse Sample No.	V	ppm Ni	Fe
1	50, 52	18, 14, 13	16
2	88	27	10
3	63	26	15
4	246, 242, 238	51.0	8.7, 9.2, 9.1
5	11	24.6	11.8
6	9	1.3	<3
7	84	27.9	13.9
8	38	10.9	<3.
9	16	21.0	12.6
10	45	17.8	30.0
11	27	16.0	9.5
12	32	20.1	15.2
13	30	19.7	8.1
14	32	18.5	25.0
15	56	30.1	19.2
16	70	33.9	12.9
17	8	8.8	11.5
18	12	10.4	33.2
19	24	7.3	4.9
20	87	23.0	13.3
21	20	9.2	12.5
22	14	12.5	10.1
23	37		17.9
24	36	46.5	16.5
25	<3	<1	4.6

Correlations between optical emission analyses results for vanadium, nickel and iron and the atomic absorption results of Table 41 are displayed in Figures 105 through 107. As can be seen, the correlations are good.

Consideration was given the use of the flameless or electro-thermal mode of atomic absorption spectroscopy for the measurement of elements at the 1 or less than 1 part per million level. However, the large number of elements that had to be measured and because it requires 5 to 10% longer per individual measurement as compared to the flame mode, the time requirements would have been prohibitive.

Arsenic and Selenium Analyses

Arsenic and selenium analyses were performed on the first 26 residual fuel oil samples. The results are presented in Table 42. At these low levels of occurrence; it would be difficult to assess the environmental significance of these elements.

Sulfur Analyses

Total sulfur was determined on the first 4 as received residual fuel oil samples by the gravimetric procedure after decomposition in an oxygen bomb and by X-Ray fluorescence. The following results were obtained:

Table 43

SULFUR CONTENTS OF AS RECEIVED SAMPLES

Oil Sample No.	Sulfur Content by Oxygen Bomb	Sulfur Content by X-ray Fluorescence
1	2.53%	2.42
2	2.41%	2.32
3	2.79%	2.70
4	2.03%	--

Table 42

ARSENIC AND SELENIUM CONTENTS OF RESIDUAL FUEL OILS

Westinghouse Sample No.	Arsenic Content (ppm)	Selenium Content (ppm)
1	0.072	<0.5
2	0.11	<0.5
3	0.14	<0.5
3 (Repeat)	0.12	0.06
4D	0.087	<0.5
5A	0.15	0.30
6	0.02	0.15
7	0.057 0.054	0.14 0.14
8	0.024	0.07
9	0.109	0.09
10	0.180	0.03
11A	0.107	0.07
12A	0.140	0.05
13	0.19	0.02
14	0.119	0.09
15	0.161	0.06
16	0.25	0.08
17	0.075	0.06
18	0.22	0.04
19	0.049	<0.01
20	0.095	0.06
21	0.044	0.31
22	0.13	0.12
23	0.073	0.14
24	0.060	0.14
25	0.036	0.17
26	0.087	0.18

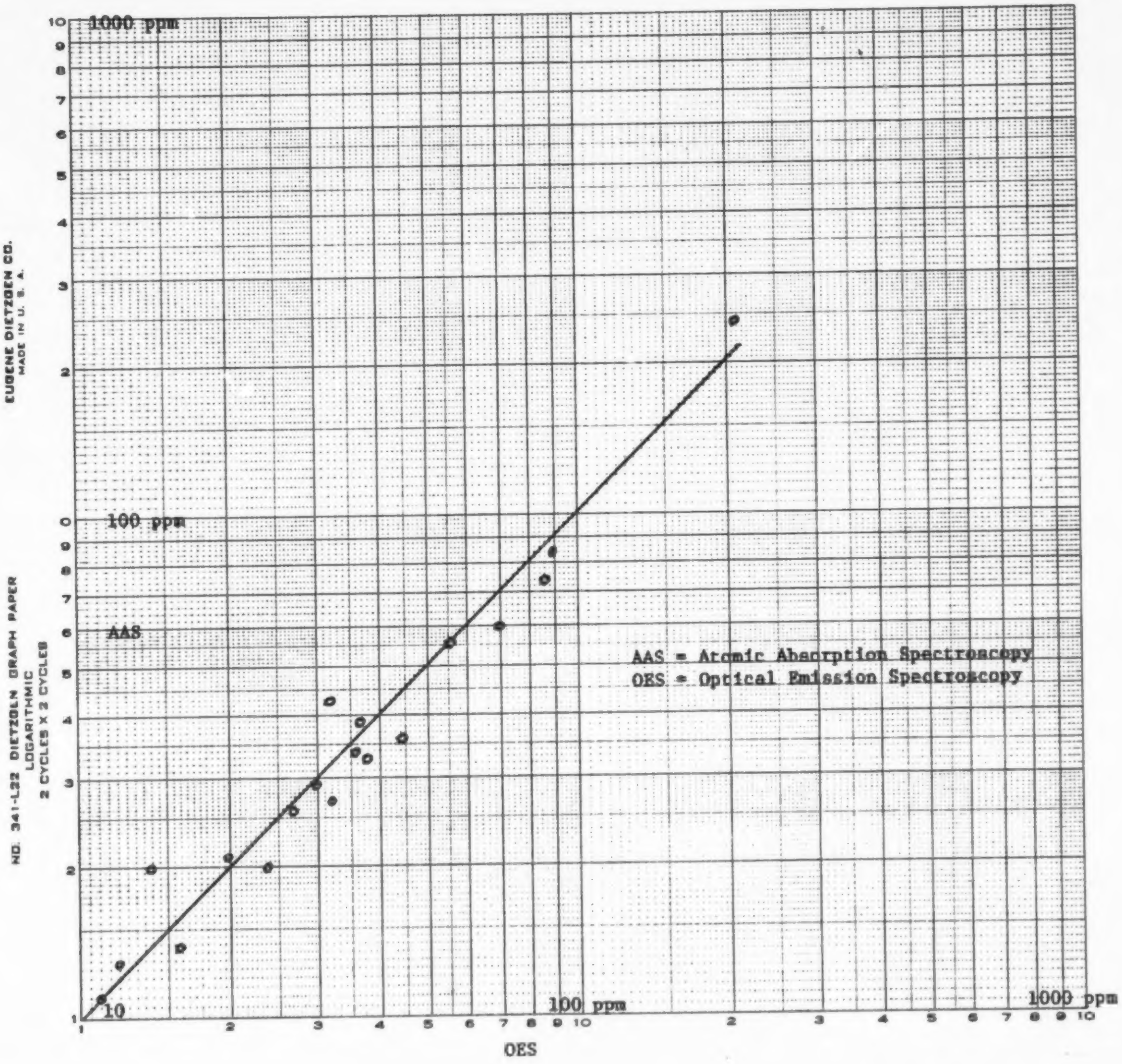

Figure 105. Correlation Plot of Optical Emission Spectroscopy (OES) Results versus Atomic Absorption Spectroscopy (AAS) Values for Vanadium

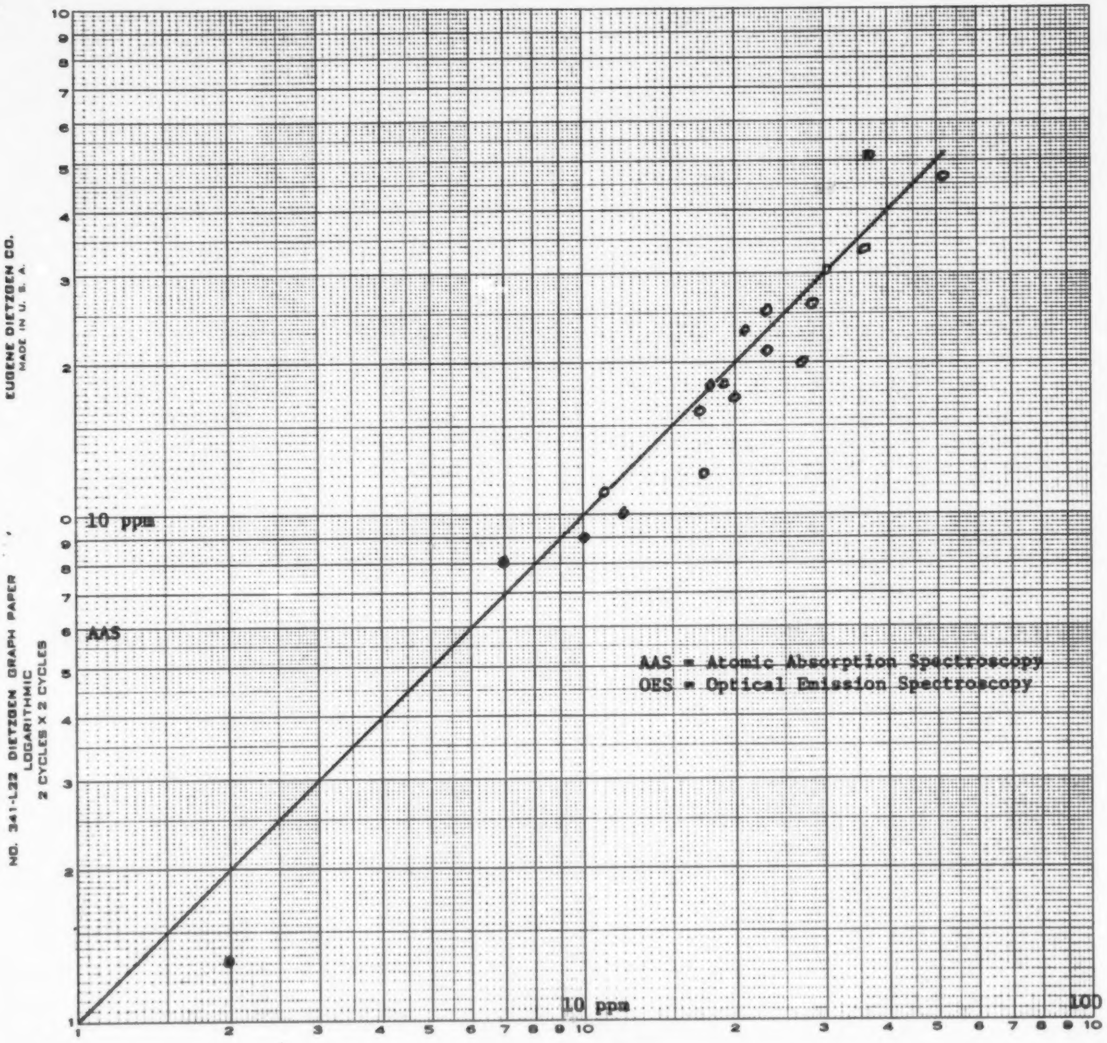

Figure 106. Correlation Plot of Optical Emission Spectroscopy (OES) Results versus Atomic Absorption Spectroscopy (AAS) Values for Nickel

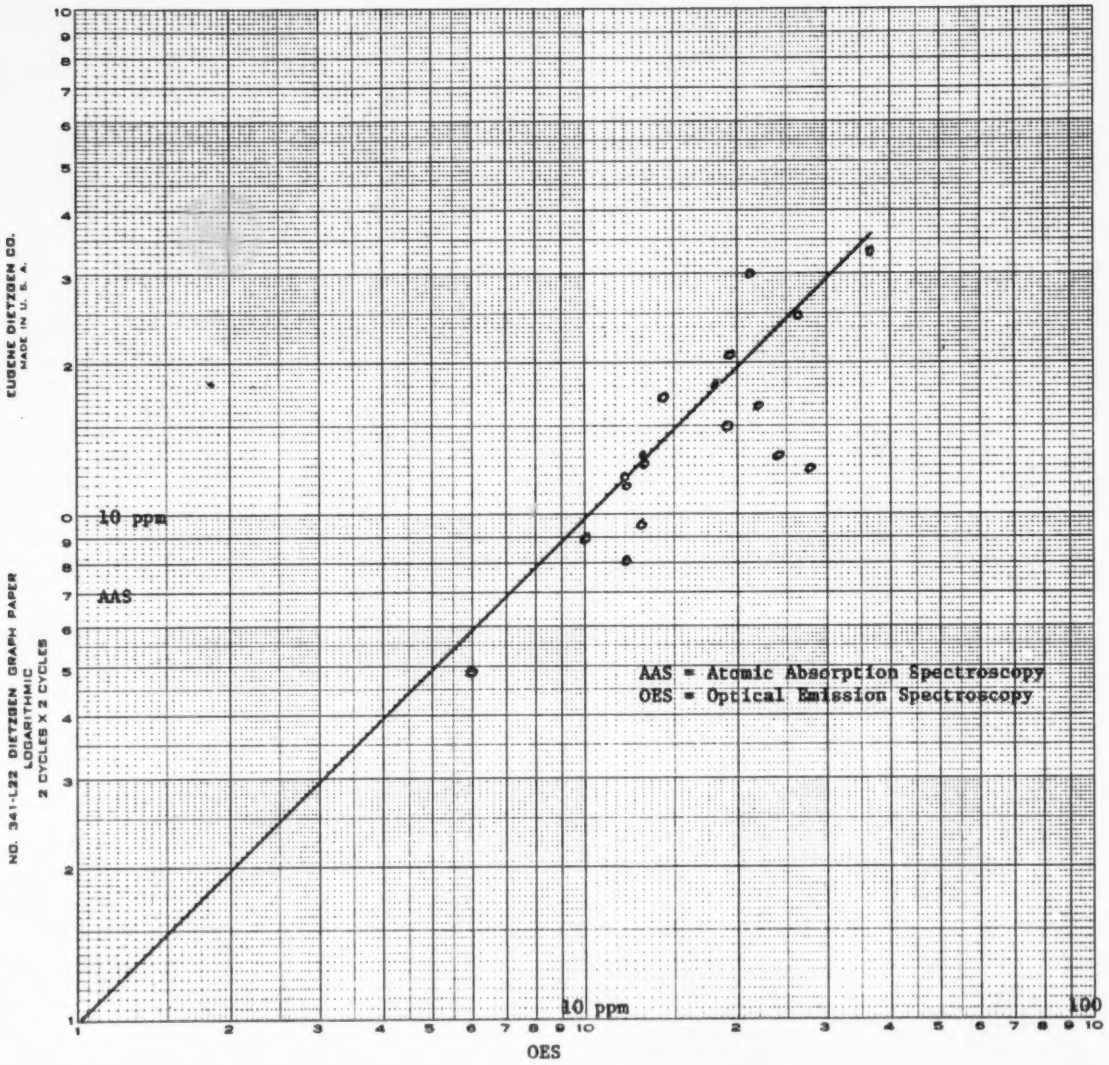

Figure 107. Correlation Plot of Optical Emission Spectroscopy (OES) Results Versus Atomic Absorption Spectroscopy (AAS) Values for Iron

Since the values agree with what is provided as a maximum sulfur content for these oils (see Table 2), there appeared to be no pressing reason for examining other as received oil samples.

During development of our column chromatography sample pre-separation fractions, the thought occurred that such separations may be followed by sulfur destination.

Sulfur analyses were done on the various fractions as obtained from the Florisil column work (i.e., the fractions, but as "finer" cuts, contained in the "florisil" column of data in Table 3 and representing about 100% recovery of the starting sample.) The results of the analyses are given in Table 44. They show that even this preliminary column work was effective in segregating compounds responsible for the sulfur content. The high sulfur "recovery" in the table data (124.1%) is probably more reflective of the accuracy used in weighing column fractions obtained rather than of quality of sulfur analyses. Finding some sulfur in each of the fractions, however, lowered the probability that sulfur content alone would be responsible in major part than any mutagenicity found in such column fractions. No further work was performed along this line.

When we started emphasizing work on analyses of mutagenically significant fractions as noted above - oil #16, Fractions 2 and 3 and oil #12, Fraction 3 we decided to see if sulfur contents of these fractions were important. The results, below, failed to disclose any such importance:

Table 45

SULFUR CONTENTS OF IMPORTANT COLUMN FRACTIONS

W Oil No.	Column Pre-Separation Fraction	Sulfur Content
16	2	1.17%
16	3	1.25%
16	4	1.50%
12	3	1.48%

Table 44

ANALYSES OF SULFUR CONTENTS - RESOLUTION OF SHELL FUEL OIL NO. 6 (W SAMPLE NO. 1) ON UNTREATED FLORISIL COLUMN

Fraction	Percent of Sample	Sulfur Content of Fraction	Sulfur Content of Fraction as % of Total Sample Sulfur
Hexane, Cut #1	2.4	0.2 %	<0.4
Cut #2	38.8	1.2	19.4
Cut #3	9.4	2.8	10.7
Cut #4	3.5	3.1	4.5
Cut #5	3.5	3.3	5.0
Cut #6	3.5	3.2	4.5
Cut #7	1.2	2.1	0.8
Cut #8	<1.0	1.7	<0.8
Hexane to 15% Acetone in Hexane	1.2	2.4	1.2
15% Acetone in Hexane, Cut #1	7.1	3.3	9.5
Cut #2	5.9	2.7	6.6
Cut #3	2.4	3.6	3.7
Cut #4	1.2	3.6	1.7
15% to 50% Acetone in Hexane	1.2	5.7	2.9
50% Acetone in Hexane	<1.0	2.7	<1.2
50% Acetone in Hexane to Acetone	1.2	2.9	1.2
Acetone	2.4	19.5	19.4
Methylene Chloride	<1.0	2.0	<0.8
Hexane Insolubles of As Received Sample	15.3	4.7	29.8
TOTALS	100.2 %		124.1 %*

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*See text.

Two experiments were performed to attempt to differentiate inorganic or elemental sulfur from organic sulfur in an oil sample. The results did not suggest pursuing this any further. Twenty grams of Oil #1 (Shell #6) were mixed in a blender with 100 ml of 1 N NaOH. The aqueous phase was separated, washed with toluene and petroleum ether. This aqueous phase was analyzed for total sulfur. The total extractable sulfur was <7 ppm. Using the same amount of oil, two samples, one containing 0.00235 g FeS (0.86 mg S) and another containing 0.00162g of elemental sulfur were extracted in a similar manner. The aqueous extract from the FeS sample contained <7 ppm S and the one from elemental sulfur contained the equivalent of 12.2 ppm, indicating that inorganic sulfur in the form of FeS or elemental sulfur are not extracted by this procedure. No attempt was made to check sulfur recovery using a known water soluble sulfur compound. In the second series of experiments, one gram of W oil sample #1 was ashed at 450°C in platinum and in graphite crucibles with and without addition of approximately 50 mg of FeS. The same amount of FeS was heated under the same conditions without oil. The results are as follows:

FeS alone in platinum crucible	90.3% S recovered
FeS alone in graphite crucible	80.6% S recovered
Oil + FeS in platinum crucible	92.9% S recovered
Oil + FeS in graphite crucible	82.4% S recovered
Oil alone in platinum crucible	<0.01% S (amount present in ash)
Oil alone in graphite crucible	<0.01% S (amount present in ash)

The conclusion is that this approach is not effective. The recovery of inorganic sulfur would not be expected to be good even in a non-oxidizing atmosphere at relatively low temperature.

Nitrogen Analyses

The total nitrogen analyses on the first three as received oil samples gave identical nitrogen values of 0.23% on all three samples. There appeared to be no reason for continuing this type of analysis. Nitrogen analyses on mutagenically important column pre-separation fractions gave the following results:

Table 46

NITROGEN CONTENTS OF IMPORTANT COLUMN FRACTIONS

W Oil Sample No.	Fraction	% Nitrogen
16	2	0.03
16	3	0.37
12	3	0.38

The "fraction 3" values would be expected from the detection of carbazole and its homologues in Fraction 16-3. No carbazole was detected in Fraction 12-3, but apparently other (aliphatic ?) nitrogen compounds find their way here - a possibly important finding. The low nitrogen content of Fraction 16-2 is also interesting, and somewhat confirmatory that polycyclic aromatics would be expected as major components of the fraction.

Other Elemental Analyses

The first 4 residual fuel oil samples were sent to the Galbraith Laboratories for elemental analyses for C, H, O, N, S, F, Cl and Br. The samples were sent ostensibly as 8 materials, with random pairing of the four samples unbeknown to the sub-contactor. The results obtained are given in Table 47.

A telephone call was made to Ms. Gail Hutchens, Executive Vice President of Galbraith Laboratories, to find out as much as possible concerning the various analytical techniques used there for these samples. The following is a summary of that conservation:

C and H - These were done by a Pregl-Ingram combustion technique followed by a gravimetric finish.

Table 47

ELEMENTAL ANALYSIS OF FOUR FUEL OILS BY GALBRAITH LABORATORIES

W Oil Sample No.	Description	% C	% H	% N	% O	% S	% F	% Cl	% Br	Total
1.	Shell #6, High Sulfur	85.82	9.81	0.33	0.93	2.66	0.001	0.079	0.009	99.64
	Fuel Oil Code - 41010	86.25	9.75	0.32	0.73	2.76	0.001	0.086	0.006	99.90
	Nov. 77									
2.	UTP @ Palmetto #6	85.25	10.57	0.23	0.57	2.24	0.001	0.056	0.004	99.43
	Fuel Oil 10/13/77	85.98	10.93	0.33	0.50	2.23	0.001	0.046	0.008	100.03
	Dow Heater #2									
3.	Exxon #6 Fuel Oil	86.43	11.26	0.30	0.58	1.52	0.003	0.040	0.002	100.13
	2.9% SX2432	86.13	10.21	0.30	0.54	2.70	0.002	0.033	0.002	99.92
	Dec. 77									
	Retest*	86.14	10.96	--	0.41	2.25 2.40	--	--	--	--
4.	Gallon Sample 4D	86.51	10.46	0.37	0.69	1.93	0.002	0.039	0.022	100.02
	Fuel Oil #6	85.45	11.18	0.29	0.78	2.34	0.001	0.039	0.014	100.09
	Dec. 77									

---

*On subsequent conversation with Galbraith Laboratories, they raised the question as to whether there were any results Westinghouse questioned. We mentioned the 1.52% sulfur value of the one analysis of sample 3. The results here were then received from Galbraith a few days later.

N - A Kjeldahl procedure using $CuSO_4$-Na_2SO_4 digestion was used. No special catalyst was employed.

S - The organic sample was combusted with O_2 in a Schöniger flask, and the resulting combustion products analyzed for sulfate by ion-chromatography.

F - The fluoride content of the combusted solution of the sample was determined by the fluoride selective-ion electrode.

Br - The bromide content of the combusted sample was determined iodometrically after selective oxidation to bromate.

Cl - Total Br and Cl was determined by $AgNO_3$ titration after Schöniger flask combustion. Cl was found by subraction of the Br content found separately.

O - Galbraith uses a novel direct method based on conversion of O to CO at high temperatures in a stream of N_2 in which the CO is measured by infrared absorption.

Their claimed accuracies are as follows:

C, H - ±0.3% (relative)
S - 0.2 - 0.3%
All others - 5-10% of the amount found.

For convenience, Galbraith results are listed with Westinghouse results in Table 48.

The halide values seemed high so analyses were repeated by Westinghouse with results as shown in Table 49. Informed that our results were lower than Galbraith's, the subcontractor suggested the chlorine contents to be re-run by him using a "different" method at no additional charge to the contract. The results are shown in Table 50. We consider our results more accurate. At the low levels found, halogen content of the residual fuel oils does not appear to be important.

Table 48

COMPARISON OF SULFUR AND NITROGEN DETERMINATIONS ON FUEL OIL SAMPLES

	% Nitrogen		% Sulfur		
Oil Sample	Galbraith (Kjeldahl)	W (Kjeldahl)	Galbraith (Schöniger)	W (X ray Fluor)	W (O_2 Bomb)
1. Shell #6 High Sulfur Fuel Oil Code 41010 Nov. 77	0.33 0.32	0.23	2.66 2.76	2.42	2.53
2. UTP @ Palmetto #6 Fuel Oil 10/13/77 Dow Heater #2	0.23 0.33	0.23	2.24 2.23	2.32	2.41
3. Exxon #6 Fuel Oil 2.9% S SX2432 Dec. 77	0.30 0.30	0.23	1.52 2.70 2.25 2.40	2.70	2.79
4. Gallon Sample 4D Fuel Oil #6 Dec. 77	0.37 0.29	—	1.93 2.34	2.05 2.02	2.03

Table 49

HALOGEN ANALYSIS OF FUEL OILS

	% (Cl + Br) as Cl		% Cl	% Cl	% F	
W Oil Sample No.	W Oxygen Bomb	W Oxygen Bomb and Distillation	W X-Ray	Galbraith	Specific Ion Electrode	Galbraith
1	<0.0010	0.0025	<0.001	0.079	<0.0002	0.001
	<0.0010	0.0031		0.086		0.001
		0.0050				
2	0.0047	0.0065		0.056	0.0016	0.001
	0.0054			0.046		0.001
3	0.0022	0.0028		0.040	<0.0002	0.003
	0.0025			0.033	<0.0002	0.002
4	0.0061	0.015	<0.006;	0.039	0.0004	0.002
	0.0068		<0.015	0.039		0.001

Table 50

VARIOUS CHLORINE VALUES OBTAINED ON FUEL OILS

	% Chlorine Content		
Oil Sample	Galbraith Results	Galbraith Re-test	Westinghouse Results
#1	0.079	0.010 0.012	0.0025 0.0031 0.0050
(Blind Duplicate)	0.086	0.005 0.005	
#2	0.056	0.013 0.012	0.0065
(Blind Duplicate)	0.046	0.010 0.010	
#3	0.040	0.012 0.014	0.0028
(Blind Duplicate)	0.033	0.008 0.008	
#4	0.039	0.015 0.017	0.015
(Blind Duplicate)	0.039	0.013 0.014	

RADIONUCLIDE ASSAY

Twenty-eight samples of fuel oil were submitted to Controls for Environmental Pollution (CEP) for radio nuclide assay. This is in response to the contract request that such data be supplied without interpretation. The results are presented as received in Table 51.

The extent of all the above experimentation can be summarized in the data of Table 52.

REPORT OF ANALYSIS

CUSTOMER Westinghouse Electric Corporation
ADDRESS 1310 Beulah Road
CITY Pittsburgh, PA 15235
ATTENTION Charles Page
INVOICE NO. 908198

Table 51

RADIONUCLIDE ANALYSIS

SAMPLES RECEIVED 12/8/78
CUSTOMER ORDER NUMBER P.O. #L-34-JP-85449
TYPE OF ANALYSIS Oil (FINAL REPORT)

Westinghouse Sample Number

Analysis	#1 pCi/g	#3 pCi/g	#4 pCi/g
Radium-226	< 0.3	< 0.3	< 0.3
Radium-228	< 0.3	< 0.3	0.4 ± 0.2
Polonium-210	0.41 ± 0.29	0.78 ± 0.34	0.62 ± 0.32
Lead-210	0.33 ± 0.19	1.62 ± 0.33	3.07 ± 0.36
Uranium-234	0.04 ± 0.03	0.09 ± 0.04	0.08 ± 0.06
Uranium-235	0.01 ± 0.03	< 0.02	< 0.04
Uranium-238	0.04 ± 0.03	0.07 ± 0.03	0.05 ± 0.05
Thorium-230	0.04 ± 0.04	0.04 ± 0.04	< 0.01
Thorium-232	0.05 ± 0.04	0.05 ± 0.03	< 0.01
Radon-222	< 0.3	0.3 ± 0.1	< 0.3

Analysis	#5 pCi/g	#6 pCi/g	#7 pCi/g
Radium-226	< 0.3	< 0.3	0.5 ± 0.4
Radium-228	< 0.3	0.4 ± 0.3	0.3 ± 0.2
Polonium-210	0.77 ± 0.35	0.47 ± 0.32	0.38 ± 0.28
Lead-210	1.74 ± 0.21	0.34 ± 0.27	1.48 ± 0.24
Uranium-234	0.04 ± 0.03	0.08 ± 0.02	0.07 ± 0.03
Uranium-235	< 0.02	< 0.02	< 0.02
Uranium-238	0.02 ± 0.02	0.03 ± 0.02	0.06 ± 0.03
Thorium-230	< 0.02	0.02 ± 0.01	0.03 ± 0.01
Thorium-232	0.02 ± 0.02	0.02 ± 0.01	< 0.01
Radon-222	< 0.3	< 0.3	< 0.3

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REPORT OF ANALYSIS

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INVOICE NO. 908198

Table 51 (Cont)

SAMPLES RECEIVED	12/8/78	CUSTOMER ORDER NUMBER	P.O. #L-34-JP-85449
TYPE OF ANALYSIS	Oil (FINAL REPORT)		

Analysis	#8 pCi/g	#9 pCi/g	#10 pCi/g
Radium-226	< 0.3	< 0.3	< 0.3
Radium-228	0.2 ± 0.2	0.4 ± 0.2	0.3 ± 0.3
Polonium-210	1.1 ± 0.4	0.87 ± 0.32	0.28 ± 0.26
Lead-210	0.39 ± 0.24	0.42 ± 0.18	0.84 ± 0.18
Uranium-234	0.25 ± 0.12	0.06 ± 0.02	0.36 ± 0.06
Uranium-235	< 0.08	< 0.02	< 0.01
Uranium-238	0.09 ± 0.09	0.03 ± 0.02	0.24 ± 0.05
Thorium-230	< 0.01	< 0.01	0.03 ± 0.02
Thorium-232	0.02 ± 0.01	< 0.01	0.05 ± 0.02
Radon-222	< 0.3	< 0.3	0.3 ± 0.2

Analysis	#11 pCi/g	#12 pCi/g	#13 pCi/g
Radium-226	< 0.3	< 0.3	< 0.3
Radium-228	< 0.3	< 0.3	< 0.3
Polonium-210	0.46 ± 0.30	0.38 ± 0.27	0.24 ± 0.29
Lead-210	0.59 ± 0.24	0.78 ± 0.20	< 0.18
Uranium-234	0.31 ± 0.12	0.04 ± 0.04	0.14 ± 0.03
Uranium-235	< 0.07	< 0.02	< 0.02
Uranium-238	0.18 ± 0.11	0.02 ± 0.04	0.07 ± 0.02
Thorium-230	< 0.01	0.02 ± 0.01	< 0.02
Thorium-232	< 0.01	< 0.01	0.01 ± 0.02
Radon-222	< 0.3	< 0.3	< 0.3

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Table 51 (Cont)

SAMPLES RECEIVED 12/8/79
CUSTOMER ORDER NUMBER P.O. #L-34-JP-85449

TYPE OF ANALYSIS Oil (FINAL REPORT)

Analysis	#14 pCi/g	#15 pCi/g	#16 pCi/g	#17 pCi/g
Radium-226	< 0.3	< 0.3	< 0.3	< 0.3
Radium-228	0.3 ± 0.1	< 0.3	< 0.3	0.1 ± 0.3
Polonium-210	0.09 ± 0.28	0.11 ± 0.28	0.10 ± 0.22	0.05 ± 0.25
Lead-210	0.33 ± 0.12	0.42 ± 0.11	0.76 ± 0.17	0.42 ± 0.16
Uranium-234	0.06 ± 0.03	0.04 ± 0.03	0.12 ± 0.08	0.04 ± 0.03
Uranium-235	< 0.02	< 0.02	< 0.06	< 0.02
Uranium-238	0.04 ± 0.03	0.01 ± 0.02	0.08 ± 0.08	0.01 ± 0.02
Thorium-230	< 0.02	< 0.01	< 0.02	< 0.02
Thorium-232	< 0.02	< 0.01	< 0.02	< 0.02
Radon-222	< 0.3	< 0.3	< 0.3	< 0.3

Analysis	#18 pCi/g	#19 pCi/g	#20 pCi/g	#21 pCi/g
Radium-226	0.3 ± 0.4	< 0.3	< 0.3	< 0.3
Radium-228	< 0.3	< 0.3	0.3 ± 0.2	< 0.3
Polonium-210	0.65 ± 0.35	0.30 ± 0.27	0.14 ± 0.24	< 0.24
Lead-210	1.30 ± 0.14	1.08 ± 0.15	0.21 ± 0.13	< 0.10
Uranium-234	0.17 ± 0.09	0.25 ± 0.16	0.12 ± 0.11	0.33 ± 0.07
Uranium-235	0.05 ± 0.06	< 0.12	< 0.05	0.01 ± 0.02
Uranium-238	0.17 ± 0.09	0.16 ± 0.15	0.05 ± 0.07	0.33 ± 0.07
Thorium-230	< 0.02	0.01 ± 0.04	< 0.01	< 0.06
Thorium-232	0.03 ± 0.02	0.01 ± 0.03	< 0.01	< 0.05
Radon-222	< 0.3	< 0.3	< 0.3	< 0.3

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Table 51 (Cont)

REPORT OF ANALYSIS

SAMPLES RECEIVED 12/8/78	CUSTOMER ORDER NUMBER P.O. #L-34-JP-85449
TYPE OF ANALYSIS Oil (FINAL REPORT)	

Analysis	#22 pCi/g	#23 pCi/g	#24 pCi/g	#25 pCi/g
Radium-226	0.3 ± 0.3	< 0.3	0.7 ± 0.3	0.3 ± 0.4
Radium-228	0.5 ± 0.1	< 0.3	0.1 ± 0.3	< 0.3
Polonium-210	0.17 ± 0.27	< 0.23	0.12 ± 0.24	0. 16 ± 0.23
Lead-210	1.23 ± 0.21	2.49 ± 0.23	0.83 ± 0.18	0.25 ± 0.13
Uranium-234	0.11 ± 0.04	0.13 ± 0.03	0.33 ± 0.06	1.06 ± 0.11
Uranium-235	0.01 ± 0.02	< 0.02	0.01 ± 0.01	0.03 ± 0.02
Uranium-238	0.05 ± 0.03	0.04 ± 0.02	0.29 ± 0.05	1.07 ± 0.11
Thorium-230	< 0.01	< 0.02	0.02 ± 0.02	< 0.02
Thorium-232	< 0.01	< 0.02	0.01 ± 0.02	< 0.02
Radon-222	< 0.3	< 0.3	0.3 ± 0.1	< 0.3

Analysis	#26 pCi/g	#27 pCi/g	#28 pCi/g	#29 pCi/g
Radium-226	1.2 ± 0.3	1.3 ± 0.4	< 0.3	< 0.3
Radium-228	< 0.3	0.3 ± 0.2	< 0.3	< 0.3
Polonium-210	0.22 ± 0.22	0.43 ± 0.22	0.12 ± 0.24	0.09 ± 0.25
Lead-210	0.36 ± 0.18	0.41 ± 0.10	1.03 ± 0.20	0.86 ± 0.15
Uranium-234	0.18 ± 0.05	0.05 ± 0.03	0.02 ± 0.02	0.27 ± 0.12
Uranium-235	< 0.02	< 0.02	< 0.02	< 0.08
Uranium-238	0.08 ± 0.04	0.03 ± 0.03	0.02 ± 0.02	0.21 ± 0.12
Thorium-230	< 0.02	0.07 ± 0.02	0.04 ± 0.02	0.02 ± 0.01
Thorium-232	< 0.01	< 0.01	< 0.01	0.02 ± 0.01
Radon-222	< 0.3	< 0.3	< 0.3	< 0.3

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Table 51 (Cont)

REPORT OF ANALYSIS

SAMPLES RECEIVED 12-8-78
CUSTOMER ORDER NUMBER PO # 34-JP-85449-A
TYPE OF ANALYSIS Oil - Carbon-14

Sample Identification	pCi/g
# 1	ND
# 3	27±89
# 4	ND
# 5	ND
# 6	ND
# 7	53±89
# 8	28±90
# 9	ND
# 10	ND
# 11	ND
# 12	ND
# 13	ND
# 14	ND
# 15	61±154
# 16	ND
# 17	83±105
# 18	ND
# 19	ND
# 20	ND
# 21	ND
# 22	81±79
# 23	ND
# 24	ND
# 25	ND
# 26	ND

ND - None Detectable

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Table 51 (Cont)

REPORT OF ANALYSIS

SAMPLES RECEIVED 12-8-78	CUSTOMER ORDER NUMBER PO # 34-JP-85449-A

TYPE OF ANALYSIS Oil - Carbon-14

Sample Identification	pCi/g
# 27	ND
# 28	ND
# 29	52±90

ND - None Detectable

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Table 52

MAJOR TYPES OF ANALYSES PERFORMED

Type of Analysis	Method of Analysis	Samples Involved
Organic	Combined, Gas Chromatography-Mass Spectroscopy	3 as Rec'd Residual Oil Samples
		23 Oils as 7 Column Pre-separation Fractions from Each
		Volatiles (to about 110°C) from 3 Oils
		7 Column Pre-separation Fractions from Dimethyl Sulfoxide Solubles of an Oil
		7 Column Pre-separation Fractions from Dimethyl Sulfoxide Insolubles of an Oil
	Mass Chromatography*	3 (critical) Column Pre-separation Fractions
	Direct Probe MS	3 (critical) Column Pre-separation Fractions
	Infra-Red Spectroscopy	36 as Rec'd Residual Oil Samples
		7 Column Pre-separation Fractions from an Oil
		10 Sub-fractions of a Critical Column Pre-separation Fraction of an Oil
		6 Fractions from Solvent Partitioning of an Oil
		7 Column Pre-separation Fractions from Dimethyl Sulfoxide Solubles of an Oil
		7 Column Pre-separation Fractions from Dimethyl Sulfoxide Insolubles of an Oil
	Observation of Ultra-violet Induced Fluorescence	All 41 Residual Oil Samples Received
		Column Pre-separation Fraction #2 from 31 Oils
		Column Pre-separation Fraction #3 from 32 Oils
		7 Column Pre-separation Fraction from an Oil
		22 Column Pre-separation Fractions from an Oil
		10 Sub-fractions from a Critical Column Pre-separation Fraction - Standard Separation
		10 Sub-fractions from a Critical Column Pre-separation Fraction - "Quick" Method
		5 Fractions from Solvent Partitioning of an Oil
		6 Column Pre-separation Fractions from Dimethyl Sulfoxide Solubles of an Oil
		6 Column Pre-separation Fractions from Dimethyl Sulfoxide Insolubles of an Oil
		55 Reference Compounds Involving Polycyclic and Heterocyclic Aromatics
Bio-Assay	Test According to Ames and with E-coli, Yeast	26 As Rec'd Oils
		6 Oils as 7 Column Pre-separation Fractions from Each
		10 Sub-fractions of a Critical Column Pre-separation Fraction
		Volatiles (to about 110°C) from 3 Oils
		4 Fractions from Solvent Partitioning of an Oil
		7 Column Pre-separation Fractions from Dimethyl Sulfoxide Solubles of an Oil

*Individual mass chromatograms reconstructed for individual masses in the mass range 140 to 320. (i.e. - 181 chromatograms per sample)

Table 52 (Cont)

Type of Analysis	Method of Analysis	Samples Involved
Bio-Assay (cont'd)	Test According to Ames and with E-coli, Yeast	7 Column Pre-separation Fractions from Dimethyl Sulfoxide Insolubles of an Oil
Inorganic Analysis	Optical Emission Spectroscopy	3 Dioxane and Toluene Partitioned Oils 25 as Rec'd Residual Fuel Oils
	Atomic Absorption Spectroscopy	26 as Rec'd Residual Fuel Oils
	Arsenic Determination	26 as Rec'd Residual Fuel Oils
	Selenium Determination	26 as Rec'd Residual Fuel Oils
	Sulfur Determination	19 Column Pre-separation Fractions from an Oil 4 Critical Oil Fractions
	Nitrogen Determination	3 Critical Oil Fractions
	Fluorine Determination	4 as Rec'd Residual Fuel Oils
	Chlorine (and/or Bromine) Determination	4 as Rec'd Residual Fuel Oils
Miscellaneous	Radionuclide Assay	28 as Rec'd Residual Fuel Oils

SECTION 7

LITERATURE SURVEY

I. INTRODUCTION

As crude oil has become increasingly expensive it has become more and more imporatnt that full utilization must be made of all products derived from the crude. One of these products is residual oil, defined as "the dark colored, highly viscous oil remaining from crude oil after the more volatile portion has been distilled off".[1] This residuum has traditionally been diluted with a lighter oil and sold cheaply for use as fuels. These "residual" fuel oils have caused many problems in the past because of their high viscosity and high solids and sulfur contents. A more recent concern is the possible health hazards caused by high concentrations of toxic metals and carcinogens in the residual oil.

The crude oil which is the source of the residual is a liquid consisting of hydrocarbons and related sulfur, oxygen and nitrogen compounds. "It was apparently formed by the decay of small marine organisms under the influence of temperature, pressure, catalysts, radioactivity and bacteria. Various geological formations trapped the crude oil in underground pools protected by overlaying mineral strata."[2]

The chemical composition of crude oil is very complicated. Although all crudes have approximately the same elemental composition,[3] they have wide variations in their chemical composition and physical properties. For example, some crudes contain up to 75% gasoline, others have none. Other crudes may yield up to 65% asphalt.[4]

The chemical composition of the residuals is even more complicated than that of the crudes for several reasons:

a) Crude oils are mixtures of many thousands of individual compounds. Almost all of these compounds may still be present in the residual oil.

b) It has been experimentally verified that the number of constituents of a petroleum fraction increases as its boiling point increases.[5]

c) The composition of the chemicals in the oil can be changed during the refining process. For example, the presence of polycyclic aromatic compounds is rare in crude oil but large amounts of such compounds are formed during pyrolysis or catalytic cracking.[6]

This report is a summary of the information available in the published literature on the availability, analysis, and hazards of residual oils.

II. THE PRODUCTION AND USE OF RESIDUAL OILS

A) Origin

The chemical composition of residual oil varies with the composition of the crude from which it is produced. The chemical composition of crude oil varies with each oil well. In some cases the composition of an individual well will even vary over its lifetime. Generally, however, it is assumed that all of the oil from each oil field is similar. In the United States there are considered to be 276 oil fields; in the world, 283 more.[7] This is a total of 559. These fields supply 294 United States refineries.[8] A complete list of oil fields is given in Appendix H. United States refineries are shown in Appendix I. A small list of crudes and residuals that are believed to be representative of all types is shown in Table 53.

Table 53

SOURCE OF SAMPLES USED BY DUEWER ET.AL.[9]

Crude Oils

1. East Texas Field, Texas
2. El Morgan, Egypt
3. Wilmington, California
4. Minas Field, Sumatra
5. Duri Field, Sumatra
6. Export, Neutral Zone, Saudi Arabia, Kuwait
7. Kuwait Export, Kuwait
8. Ceuta, East Shore Lake Maracaibo, Venezuela
9. Mesa, Orinoco Basin, Venezuela
10. Timbalier Bay, Offshore Louisiana
11. Sarir, Sirte Basin, Libya
12. Agha Jari, Iran
13. Orito, Putamayo Basin, Columbia
14. Muwait, Export Blend
15. Arabian Light, Export Blend
16. Ward-Estes, North Field, Texas
17. Goldsmith Field, Texas
18. Kelly-Snyder Field, Texas
19. Sprayberry (Trend Area), Texas
20. Headlee, Texas

Residual Fuel Oils

21. Ceuta, East Shore, Lake Maracaibo, Venezuela
22. Mesa, Orinoco Basin, Venezuela
23. Timbalier Bay, Offshore Louisiana
24. Sarir, Sirte Basin, Libya
25. Minas Field, Sumatra

Table 53 (Cont)

26. Agha Jari, Iran
27. Orito, Putamayo Basin, Columbia
28. Muwait, Export Blend
29. Arabian Light, Export Blend
30. Philadelphia No. 6
31. Santa Fe No. 6
32. Uengref Menez Bunker C
33. Port Arthur No. 6
34. Caribbean 220 vis.
35. Uengref Menez No. 6
36. Zakum
37. Nigerion
38. Caleinda
39. Oficina
40. Sumatron

B) Processing History

The chemical structure of the residuum is defined by the refining history of the crude as well as its origin. Refineries are designed so that processes and process parameters can be varied to meet market conditions for the petroleum products. In winter, for example, larger quantities of fuel oils are produced at the expense of the lighter fractions. Thus, in order to characterize a residue, the crude source, refining method, and refining parameters should be known. The chemical structure of the residue is often altered greatly by the methods used to remove the more useful materials. For the process used in each refinery, see Appendix I.

C) Chemical Composition of Original Crude Base

Among crude oils of similar types, classsification in terms of specific gravity is effective since it gives a rough idea of the gasoline or kerosene content as against more dense constituents. This system fails as soon as crudes of different types are compared. It is necessary to first make a classification based on chemical composition before the gravity scale can be used. The classification is determined by the "base" or residual fraction of the oil. This base can be waxy, asphaltic, or a mixture of the two. An early method of classification was the triangular diagram (Figure 108) of Cruse and Stephens.[10]

A more quantitative approach was that of Von Nes and Von Weston[11] as shown in Figure 109. Here the percentages of total carbon present as paraffinic chains, aromatic rings and naphtenic rings were determined by structural group analysis of the crude oil. The points for the crudes are then plotted with triangular coordinates, the apices for the triangle being 100% paraffins, naphthenes, and aromatics. From a more practical standpoint, a refiner is likely to classify crudes on the basis of gravity, sulfur content, pour point and the carbon residue value of some specified distillation residue.

Dwg. 6439A17

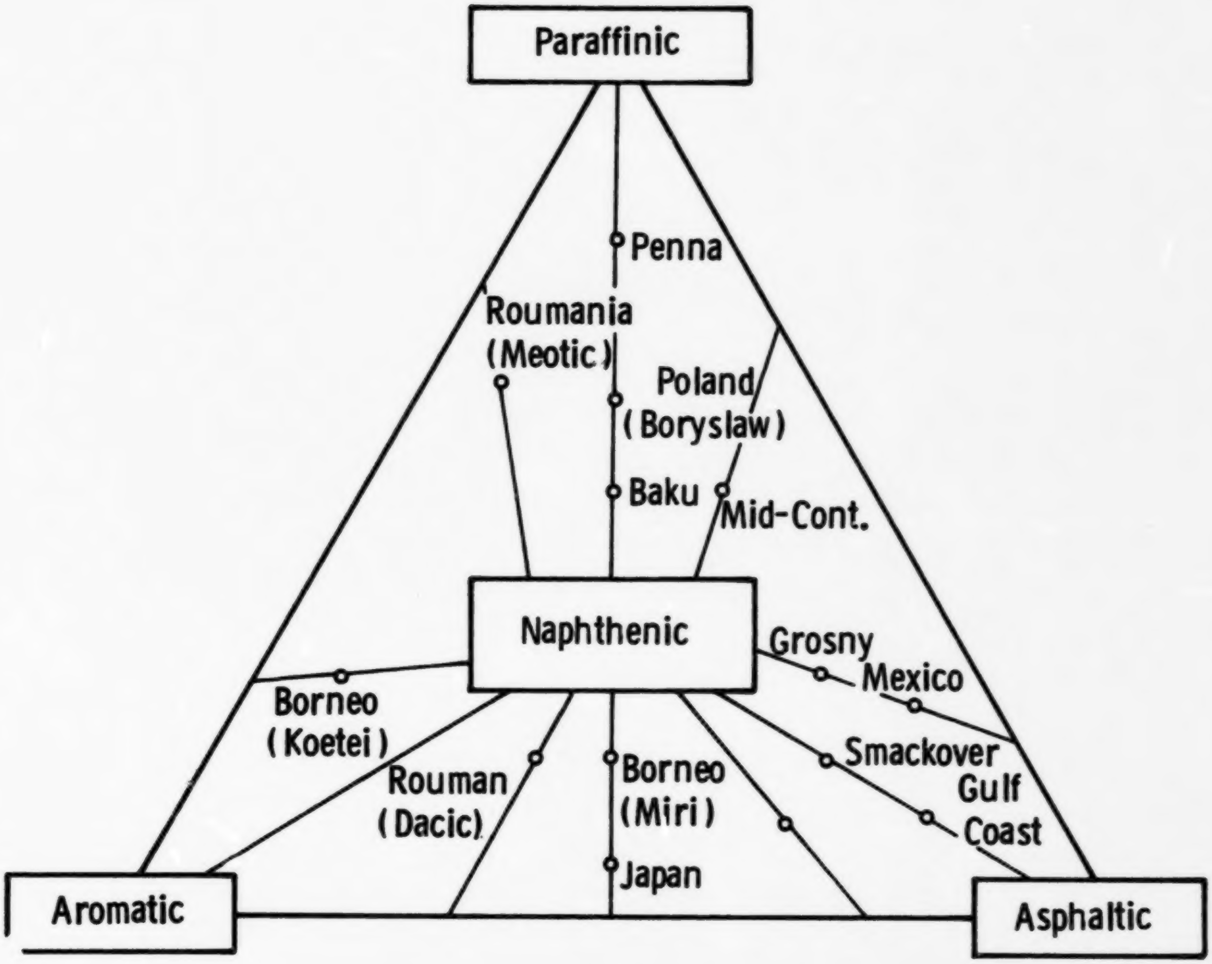
Paraffinic
Penna
Roumania
(Meotic)
Poland
(Boryslaw)
Baku
Mid-Cont.
Naphthenic
Grosny
Mexico
Borneo
(Koetei)
Rouman
(Dacic)
Borneo
(Miri)
Japan
Smackover
Gulf
Coast
Aromatic
Asphaltic

Figure 108. Classification of Crude Oils from "Chemical Technology of Petroleum", 2nd E., 1942-43[10]

Dwg. 6439A14

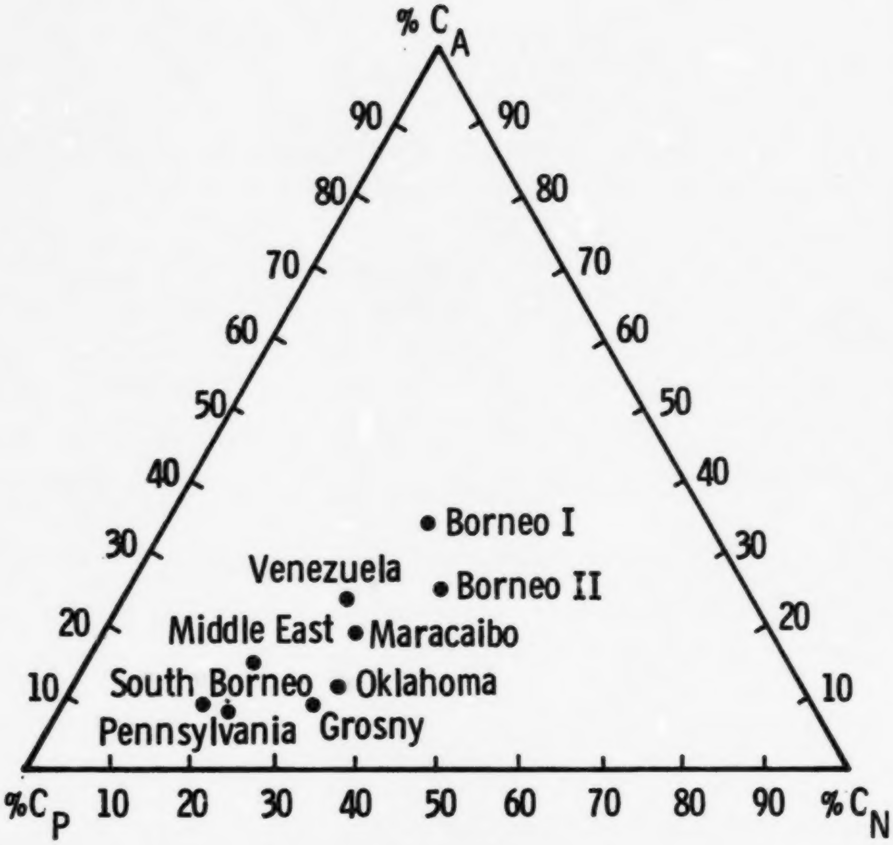

Figure 109. Classification of Crude Oils from "Constitution of Mineral Oils", 1951[11]

D) Quantities of Residual Oils Available

Although the production of residual oil varies with market conditions, the United States production for one week was 1,646,000 barrels of 42 gallon barrels. In that same week, 1,389,000 barrels were imported.

E) Projected End Use

The total use of residual oil in the United States has increased year by year as is shown in Table 54.[13] The end uses of the oil are shown in Table 55.[12] It can be seen that the percentages of each use, with one exception, declined. This exception was electric power generation. This trend was caused by the switchover from coal to oil in these plants in order to meet anti-pollution requirements.

In the future the use of oil for space heating and electric power generation should decline rapidly because of the high cost of the oil and new developments in the cleaner combustion of coal. Transportation and manufacturing use will probably remain about the same.

The definition of residual oil used in Tables 54 and 55 is as follows: Tapped crude oil obtained in refining operations includes ASTM Grade No. 5 and 6, heavy diesel, Navy Special and Bunker C oils used for generation of heat and/or power. It also includes acid sludge and pitch used for refinery fuels.

III. CHARACTERIZATION OF RESIDUAL OILS

In general, the constituents of the residual oils are saturates (hydrocarbons, cycloparaffins), aromatics (mono, di and polynuclear) tars and asphaltenes. Some idea of the complexity possible in these materials can be found in the consideration of a $C_{20}H_{42}$ hydrocarbon. Although it is near the lower limit of what are know as high molecular petroleum compounds, it can have 366,000 isomers. In addition to these compounds there are innumerable possible linkages to aromatics,

Table 54

TOTAL USE OF PETROLEUM PRODUCTS IN THE U.S.

TOTAL USE IN U.S.
(thousands of barrels)

Year / Petroleum Product	1973	1972	1971	1970	1969	1968	1967	1966	1965
Aviation Jet Fuel	372,129	373,303	357,392	349,146	366,559	349,474	305,759	232,078	
Distillate Oil	1,130,657	1,074,874	971,320	927,250	900,300	873,100	829,200	809,713	786,891
Residual Oil	1,019,934	925,647	838,044	804,287	722,529	669,484	639,539	613,972	574,118
Ethane and Propane	445,377	451,047	402,792	377,964	377,535	319,719	277,182		

Table 55

RESIDUAL OIL - CATEGORIES OF END USE[13]

YEAR	TRANSPORTATION USE	ELECTRIC POWER GENERATION USE	SPACE HEATING USE	MANUFACTURING INDUSTRY USE	N.E.C. (Not elsewhere classified)
65	.1350	.2000	.3480	.2280	.0880
66	.1260	.2290	.3450	.2150	.0850
67	.1350	.2480	.3640	.1770	.0770
68	.1370	.2760	.3260	.1960	.0650
69	.1200	.3430	.3060	.1770	.0550
70	.1140	.3880	.2860	.1660	.0450
71	.0950	.4440	.2510	.1680	.0420
72	.0850	.4700	.2570	.1510	.0360
73	.0910	.4990	.2340	.1490	.0280
74	.0900	.5340	.2170	.1390	.0200
75	.0840	.5720	.1990	.1310	.0160

napthenics, olefins, and nonhydrocarbons which may have sulfur, oxygen, or nitrogen linkages. The tars are hetero-organic, high molecular weight (500-1200) compounds which contain, in addition to carbon and hydrogen, many other elements including sulfur, oxygen, nitrogen and metals. Tars contain the bulk of sulfur, oxygen and nitrogen compounds in crudes. The highest molecular weight compounds in the residue are the asphaltenes. They are similar to the tars but have a molecular weight 2-3 times higher. Some of the trace metals that have been found in oils include Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Pb, Mg, Mn, Mo, Ni, Sb, Se, Sn, T, V and Zn.

A large number of analytical techniques have been used for the separation and identification of the constituents of residual oils. These include infrared spectroscopy (IR), ultraviolet spectrophotometry (UV), nuclear magnetic resonance spectroscopy (NMR), atomic absorption spectroscopy (AA), gas liquid chromatography (GLC or GC), liquid chromatography (LC), gel permeation chromatography (GPC), mass spectrometry (MS), elution chromatography, neutron activation, and combinations of all of these. Many of the methods were developed in response to the need for the identification of the source of oil spills. Some investigators did not attempt to separate and identify the individual constituents of the material but used methods of physiocochemial analysis to determine the characteristics of the mult-component mixture.[14]

Probably the most common method of separating heavy petroleum is elution chromatography (also called liquid-solid adsorption chromatography). A few of these methods are listed in Table 56; others will be found in Appendix J.

Another traditional method for separating heavy oils is distillation. This was investigated by Rossini, et.al.[15] in API Research Project 6. Isolation and identification of aromatic compounds on this project was also carried out by Yew and Mair.[16] A simulated distillation method

Table 56

ELUTION CHROMATOGRAPHIC METHODS

Date	Elution Solvent	Elution Column	Matrix	Separation Achieved	Detection Method Used	Pretreatment	Reference
Dec. 1956	Cyclo Hexane, n-Hexane, Benzene	Silica Gel	Oil, Lubricating	Saturates-Aromatics	Mass Spec, U.V. after Thermal Diffusion	Distillation	32
Feb. 1966	n-Pentane	Florisil	Asphalts	30 Fractions	U.V., I.R., Mass. Spec. after Thermal Diffusion	Asphaltene Separation, Sulfuric Acid	33
Dec. 1967	Pentane, $C\ Cl_4$, $CH\ Cl_3$ Methanol Pyridine (Differing Polarity)	Diatomaceous Earth	Asphalts	Saturates-Aromatics	N.M.R.	--	25
Dec. 1967	n-Pentane, Benzene Methanol Isopropylamine	Cation Exchange Resin	250-500°C Pet. Fraction	Sulfoxides from oil	I.R., N.M.R., Mass. Spec.	Distillation	26
Dec. 1967	n-Pentane, n-Hexane, Dichloro methane, Tetrahydro furane, DCM in Methanol	Alumina	Heavy Petroleums	Seven Major Groups	U.V.	Dissolved in 2:1 DCM: Cyclo Hexane	27
Sept. 1971	n-Pentane Benzene Methanol, Chloroform, Meth. Chloride, 1,2-Dichloro-Ethane (API Project 60)	Anion Exchange Resin, Cation Exchange Resin, Ferric Chloride-Kaolin Chromosorb, Silica Gel	Heavy End Distillates	Five Fractions Acid, Base, Neutral Nitrogen, Saturates, Aromatics	Mass. Spec.	Vacuum Distillation	28
May 1972	As above plus Diethyl Ether	Silica Gel-Aluminum	Heavy End Distillates (330-540°C)	Saturates, Monoaromatics, Diaromatics, Polaromatics	Mass. Spec., U.V.	Anion, Cation, Ferric Chloride Treatment	29

Table 56 (Cont)

Date	Elution Solvent	Elution Column	Matrix	Separation Achieved	Detection Method Used	Pretreatment	Reference
Dec. 1972	n-Hexane, Cyclohexane, Chloroform, Methanol	Alumina	Heavy Distillate	Mono-, Di-, Tri-, and Poly-Aromatics	U. V.	Anion, Cation, Ferric Chloride Treatment	34
July 1973	Same as API Project 60	Silica-Alumina	Heavy Distillate (370-535°C)	Saturates, Mono-, Di-, and Poly-Aromatics	Mass. Spec., N.M.R.	Distillation, Anion, Cation, Ferric Chloride Treatment	30
March 1977	n-Heptane	μ Porasil (Liquid Chromo.)	Residuals	Saturates, Aromatics	Ref. Index, Moving Wire	Various	31

using gas chromatographic techniques was reported by Worman and Green.[17] Helm[18] used a Jena Glaswerke all-glass still for the molecular distillation of 5.5 kg of asphalt.

Other separation systems that have been used are inverse gas-liquid chromatography[19,20] in which an asphalt under test serves as the stationary phase in a GLC column. It is analyzed by observing the retention behavior of volatile test compounds. Some other asphalt extraction procedures are shown in Figure 110, 111 and 112 . Several procedures[21,22,23] have been patented for removing metallic impurities from residuals. These involve washing the oil with various organic and inorganic reagents. Drushel and Somers[24] separated sulfur compounds by several methods. The first involved oxidizing the compounds to sulfones with hydrogen peroxide. They were separated by liquid chromatography and identified by mass spectrometry. Other methods involved oxidation to sulfoxides and dealkylation over alumina.

Some of the separation methods shown in Table 56 are also interesting for the methods used to identify the separated fractions. Ramsey[25] evaluated the usefulness of NMR in asphalt characterization. Okuno[26] et.al employed mass and nuclear magnetic reasonance spectroscopy to suggest that sulfoxides have saturated cyclic structures. Middleton[27] and Jewell[28] used U.V. detection at several different wavelengths. Retention data for standard activity Merck Alumina as determined by Middleton are shown in Table 57. Hirsch[29] et.al. adapted radio-tracer techniques to determine the point of elution of selected compounds. Aromatic mixtures were analyzed by gel permeation chromatography, mass spectrometry and NMR. The Bureau of Mines procedure described by Dooley[30] uses mass spectral data for quantitative determination of aromatics and sulfur compounds. Drushel[31] utilized a liquid chromatograph with a moving wire detector to measure the saturates and aromatics in petroleum residues. Helm[18] used IR and reported that methylene chloride as a solvent permitted the resolution of the 1700 cm^{-1} carbonyl bond into

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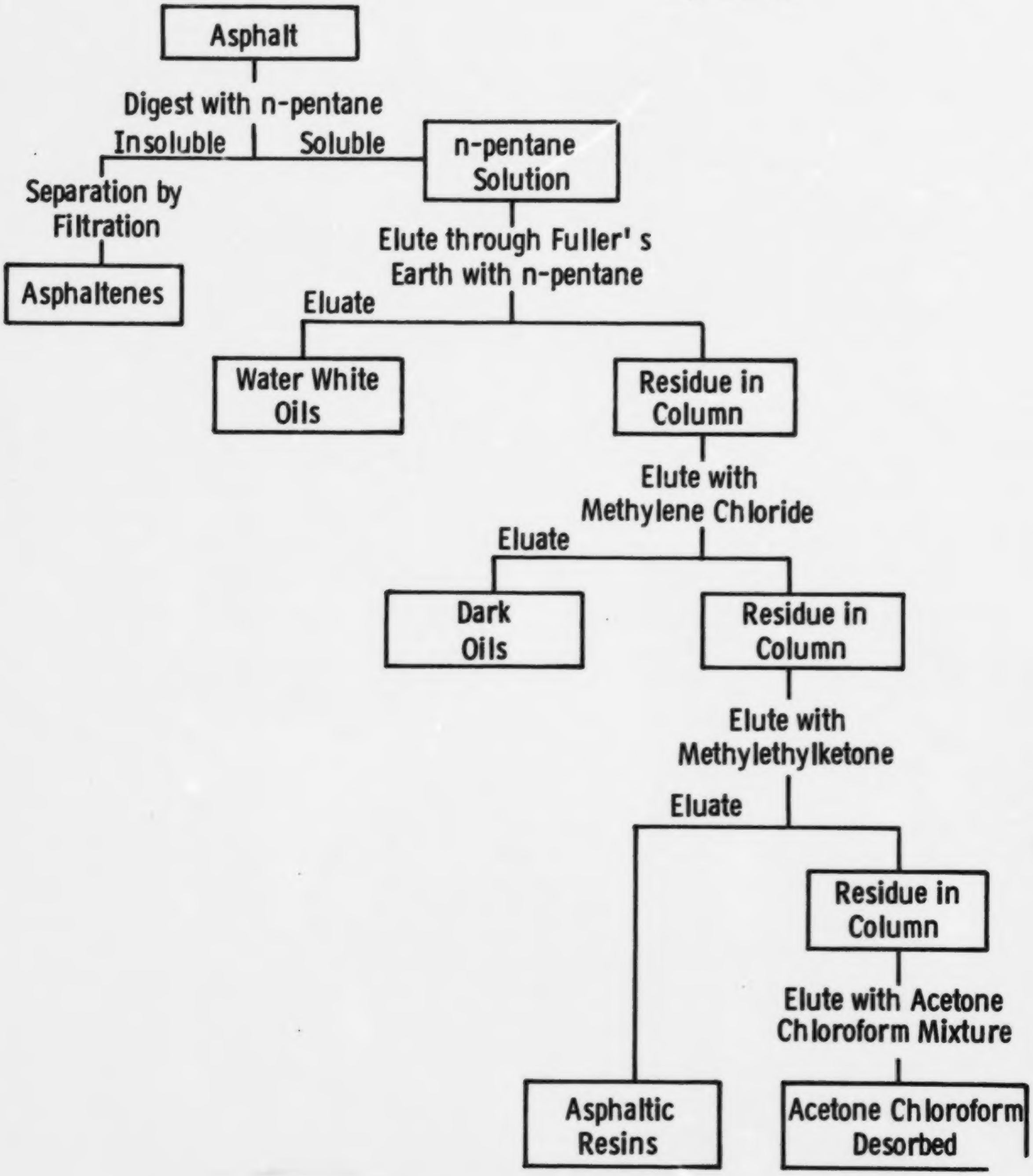

Asphalt
Digest with n-pentane
Insoluble
Soluble
n-pentane Solution
Separation by Filtration
Asphaltenes
Elute through Fuller's Earth with n-pentane
Eluate
Water White Oils
Residue in Column
Elute with Methylene Chloride
Eluate
Dark Oils
Residue in Column
Elute with Methylethylketone
Eluate
Residue in Column
Elute with Acetone Chloroform Mixture
Asphaltic Resins
Acetone Chloroform Desorbed

Figure 110. Kleinschmidt Method of Asphalt Fractionation

Dwg. 6439A16

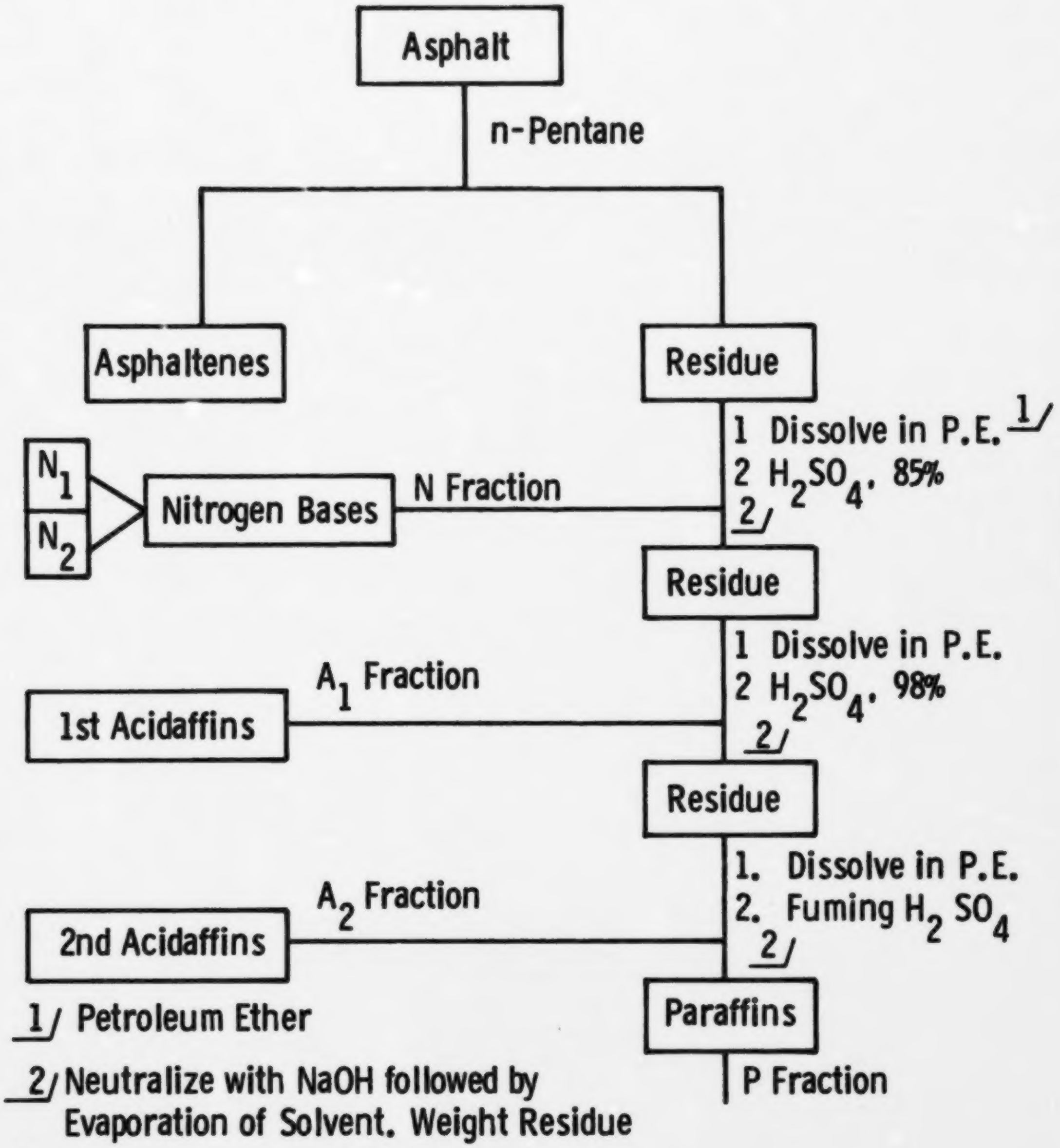
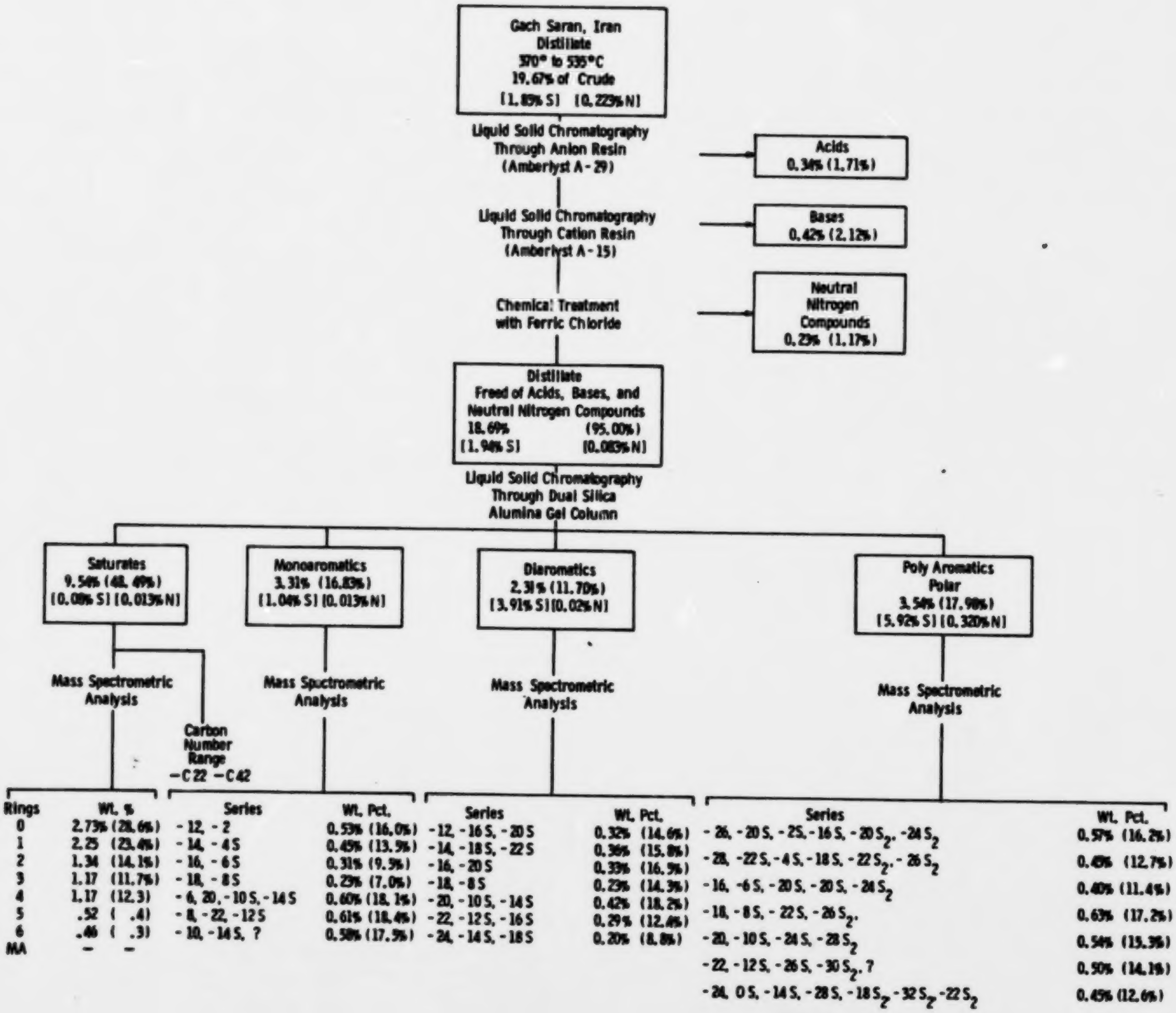

Asphalt
n-Pentane
Asphaltenes
Residue
1 Dissolve in P.E. 1/
2 H_2SO_4, 85%
2/
N_1
N_2
Nitrogen Bases
N Fraction
Residue
1 Dissolve in P.E.
2 H_2SO_4, 98%
2/
A_1 Fraction
1st Acidaffins
Residue
1. Dissolve in P.E.
2. Fuming H_2SO_4
2/
A_2 Fraction
2nd Acidaffins
Paraffins
P Fraction
1/ Petroleum Ether
2/ Neutralize with NaOH followed by Evaporation of Solvent. Weight Residue

Figure 111. Rostler-Sternberg Method of Asphalt Group Analysis

Dwg. 2618C67


Gach Saran, Iran
Distillate
370° to 535°C
19.67% of Crude
[1.85% S] [0.223% N]
Liquid Solid Chromatography
Through Anion Resin
(Amberlyst A-29)
Acids
0.34% (1.71%)
Liquid Solid Chromatography
Through Cation Resin
(Amberlyst A-15)
Bases
0.42% (2.12%)
Chemical Treatment
with Ferric Chloride
Neutral
Nitrogen
Compounds
0.23% (1.17%)
Distillate
Freed of Acids, Bases, and
Neutral Nitrogen Compounds
18.69% (95.00%)
[1.94% S] [0.083% N]
Liquid Solid Chromatography
Through Dual Silica
Alumina Gel Column
Saturates
9.54% (48.49%)
[0.08% S] [0.013% N]
Monoaromatics
3.31% (16.83%)
[1.04% S] [0.013% N]
Diaromatics
2.31% (11.70%)
[3.91% S] [0.02% N]
Poly Aromatics
Polar
3.54% (17.98%)
[5.92% S] [0.320% N]
Mass Spectrometric
Analysis
Carbon
Number
Range
-C22 -C42
Mass Spectrometric
Analysis
Mass Spectrometric
Analysis
Mass Spectrometric
Analysis

Rings	Wt. %
0	2.73% (28.6%)
1	2.25 (23.4%)
2	1.34 (14.1%)
3	1.17 (11.7%)
4	1.17 (12.3)
5	.52 (.4)
6	.46 (.3)
MA	— —

Series	Wt. Pct.
- 12, - 2	0.53% (16.0%)
- 14, - 4 S	0.45% (13.9%)
- 16, - 6 S	0.31% (9.5%)
- 18, - 8 S	0.23% (7.0%)
- 6, 20, - 10 S, - 14 S	0.60% (18.1%)
- 8, - 22, - 12 S	0.61% (18.4%)
- 10, - 14 S, ?	0.58% (17.5%)

Series	Wt. Pct.
- 12, - 16 S, - 20 S	0.32% (14.6%)
- 14, - 18 S, - 22 S	0.36% (15.8%)
- 16, - 20 S	0.33% (16.9%)
- 18, - 8 S	0.23% (14.3%)
- 20, - 10 S, - 14 S	0.42% (18.2%)
- 22, - 12 S, - 16 S	0.29% (12.4%)
- 24, - 14 S, - 18 S	0.20% (8.8%)

Series	Wt. Pct.
- 26, - 20 S, - 2S, - 16 S, - 20 S_2, - 24 S_2	0.57% (16.2%)
- 28, - 22 S, - 4 S, - 18 S, - 22 S_2, - 26 S_2	0.45% (12.7%)
- 16, - 6 S, - 20 S, - 20 S, - 24 S_2	0.40% (11.4%)
- 18, - 8 S, - 22 S, - 26 S_2.	0.63% (17.2%)
- 20, - 10 S, - 24 S, - 28 S_2	0.54% (15.3%)
- 22, - 12 S, - 26 S, - 30 S_2, ?	0.50% (14.1%)
- 24, 0 S, - 14 S, - 28 S, - 18 S_2, - 32 S_2, - 22 S_2	0.45% (12.6%)

Figure 112. Bureau of Mines Method

Table 57

RETENTION DATA FOR STANDARD ACTIVITY MERCK ALUMINA

		Retention, ml
I.	Saturates fraction	0 to 150 ± 15
	n-Tricosane	8
	Isoparaffins (∿450 mw)	∿10
	1,3-Dicyclopentyl-2-dodecyl pentane	8
	Perhydropyrene	8
II.	MNA + DNA oil fraction	151 to 360 ± 15
	1,2,3,4,5,6,7,8-octaphydro-anthracene	165
	2-n-Butyl-1-hexylindane	195
	1-Phenylpentadecane	210
	2-(Ar) decyltetralin	215
	1,4-Dimethyl-2-(3,7-dimethyl-octyl)-benzene	215
	Anylbiphenyl (tech)	255
	1,1-Diphyltetradecane	355
III.	PNA oil fraction	361 to 630 ± 15
	n-Tetradecylnaphthalene	440
	2,6-Dioctylnaphthalene	445
	Di-t-butylphenanthrene	500
	1,1,2-Triphenylethane	520 + 540
	1,1,4,4-Tetraphenyl-1,3-butadiene	575

Table 57 (Cont)

IV.	Soft resin fraction	631 to 690 ± 15
	9-Dodecylphenanthene	650
	Dibenzothiophrene	645
	Benzyl-1-Naphthyl ether	660
	9,10-Dimethyl-1,2-benzanthracene	660
V.	Hard resin fraction	691 to 870 ± 15
	4-Acetyl-O-terphenyl	730
	Benz(α)anthracene-7,12-dione	735

three discrete bands at 1740, 1700 and 1655 cm^{-1}. For the measurement of sulfur compounds after gas chromatography, Drushel and Somers[24] employed a microcoulemetric sulfur detector.

Many techniques have been developed to characterize a sample (usually from oil spills) as asphalt, heavy residual oil or crude oil residue. Kawahm and Ballinger[35] analyzed oil slicks by the method of ratios of infrared absorbance at different wavelengths. Ratios for various asphalts and residual oils were calculated. Other infrared techniques used by Kwahara[36,37] were statistical discriminant function analysis and infrared absorbance ratio (R.I.A.). Kwahara and Yang[38] also described a technique using infrared and gas chromatography data for characterization and identification of crude oils, heavy residual oils and asphalts. Smith et.al.[39] determined the infrared spectra of asphalts from six crude oil sources. Gary and Muth[40] described a simple method for characterization of oils that uses a flame ionization detector for hydrocarbon and a flame photometric detector for sulfur-containing compounds. Thruston and Knight[41] developed a method comparing the fluorescence intensities and wave number ratios for various oils.

Duewer et.al.[9] used pattern recognition and neutron activation analysis on 20 crude oils and 20 residual fuel oils. The sources of samples analyzed are listed in Table 53. These oils were chosen to be representative of materials shipped on U.S. waterways. A total of 22 elements (Table 58) was detected with an average of 10 elements detected per oil category.

Trace metals in oils are generally determined by atomic absorption. Vigler[42] and Gaylor analyzed for 25 metals in the range of 1 ppb to 5 ppm. Terrell[43] in a recent review of "Metals in Oils", lists 91 papers, 35 of which involve atomic absorption. Atomic fluorescence, as reported by Johnson et.al.[44] is also a promising technique for multi-element analysis. Braier and Eppolito[45] reviewed instrumental methods for the determination of trace metals in petroleum.

Table 58

ELEMENTS DETECTED BY DUEWER ET.AL.[9]

Elements	Conc. in ppm
Na	54.89
Al	2.088
S	11870.
Cl	94.92
V	48.25
Mn	0.09758
Co	0.2621
Ni	20.77
Zn	4.359
Ga	0.07844
As	0.07084
Br	0.5611
Sr	0.03493
Mo	0.1450
Ir	0.000105
Sn	0.1921
Sb	0.006205
I	0.2524
Ba	0.3086
La	0.01839
Sm	0.000470
Au	0.000024

Lumpkin[46] employed mass spectrometry for group-type analysis of high boiling compounds by determination of characteristic mass groupings. Gallegos[47] used high resolution mass spectrometry to analyze heavy petroleum stocks without preliminary separation. The high resolution mass spectrometric group-type results of distillation fractions from three crude sources are shown in Figure 113.[26] Figure 114 shows a segment from a high resolution mass spectrum of the 800-950°F fraction of Arabian crude with a list of the 19 components.[30]

Appendix K is a bibliography of review articles on the separation and identification of hazardous materials in petroleum.

IV. HAZARDS ASSOCIATED WITH CONSTITUENTS OF RESIDUAL OILS

The health problems caused by petroleum compounds have been known for some time. Mineral oil was believed to be carcinogenic over one hundred years ago.[48] The hazards of the oils fall into two classes. The first is the carcinogenicity, teratogenicity, and mutagenicity of the organic chemcials present in the oil. The second hazard is the presence of trace inorganic materials. Although the concentration of these is very low in the crude oil, they become more concentrated in the residuum as the refining process progresses.

A survey by Hueper[49] in 1942 showed all of the evidence linking petroleum factors to tumors applied to products that were produced before the advent of modern catalytic refining processes. Somewhat later (1952) Diety et.al.[50] conducted an extensive survey on the carcinogenic properties of high boiling petroleum products. Using a measure of tumor potency devised by Blanding[51] they found that catalytic cycle stocks and related products boiling above 700°F have high tumor potency values. This potency is associated entirely with the aromatic components. Crude residual were completely devoid of potency. A 1968 report[52] showed that all of the biologically active materials boiled between 662°F and 799°F (350-420°C). No single compound was found to be highly potent but the

	Liquid volume %							
	Arabia			California			Louisiana	
	500°– 650° F	650°– 800° F	800°– 950° F	500°– 650° F	650°– 800° F	800°– 950° F	500°– 650° F	650°– 800° F
Saturates								
Paraffins	38	21	15	2	2	3	7	4
Monocycloparaffins	7	7	7	18	9	5	21	11
Dicycloparaffins	5	4	7	15	9	8	15	11
Tricycloparaffins	3	2	5	11	8	14	11	9
Tetracycloparaffins	3	3	3	6	10	15	9	11
Pentacycloparaffins	2	4	3	4	4	6	3	8
Hexacycloparaffins	0	0	5	0	4	5	0	3
Total saturates	58	41	45	56	46	56	66	57
Aromatics								
Alkylbenzenes	13	8	10	7	7	3	7	5
Benzocycloparaffins	4	2	4	9	7	4	6	4
Benzodicycloparaffins	3	2	2	10	5	4	9	4
Naphthalenes	4	0.6	0.7	5	2	2	4	2
Acenaphthenes	1	1	1	6	5	4	3	2
Fluorenes	1	4	0.4	5	6	4	4	5
Phenanthrenes	2	10	6	0	12	7	0	13
Pyrenes	0	5	11	0	6	9	0	4
Chrysenes	0	2	3	0	0	3	0	0
Total aromatics	28	35	38	42	50	40	33	39
Sulfur compounds								
Benzothiophenes	9	6	7	2.4	2	2	0.8	0.6
Dibenzothiophenes	5	19	6	0.1	2	1	0.6	3
Naphthobenzothiophenes	0	0	4	0	0	0.7	0	0
Total sulfur compounds	14	24	17	2	4	4	1	4

Figure 113. High Resolution Mass Spectrometer Group-Type Results[26]

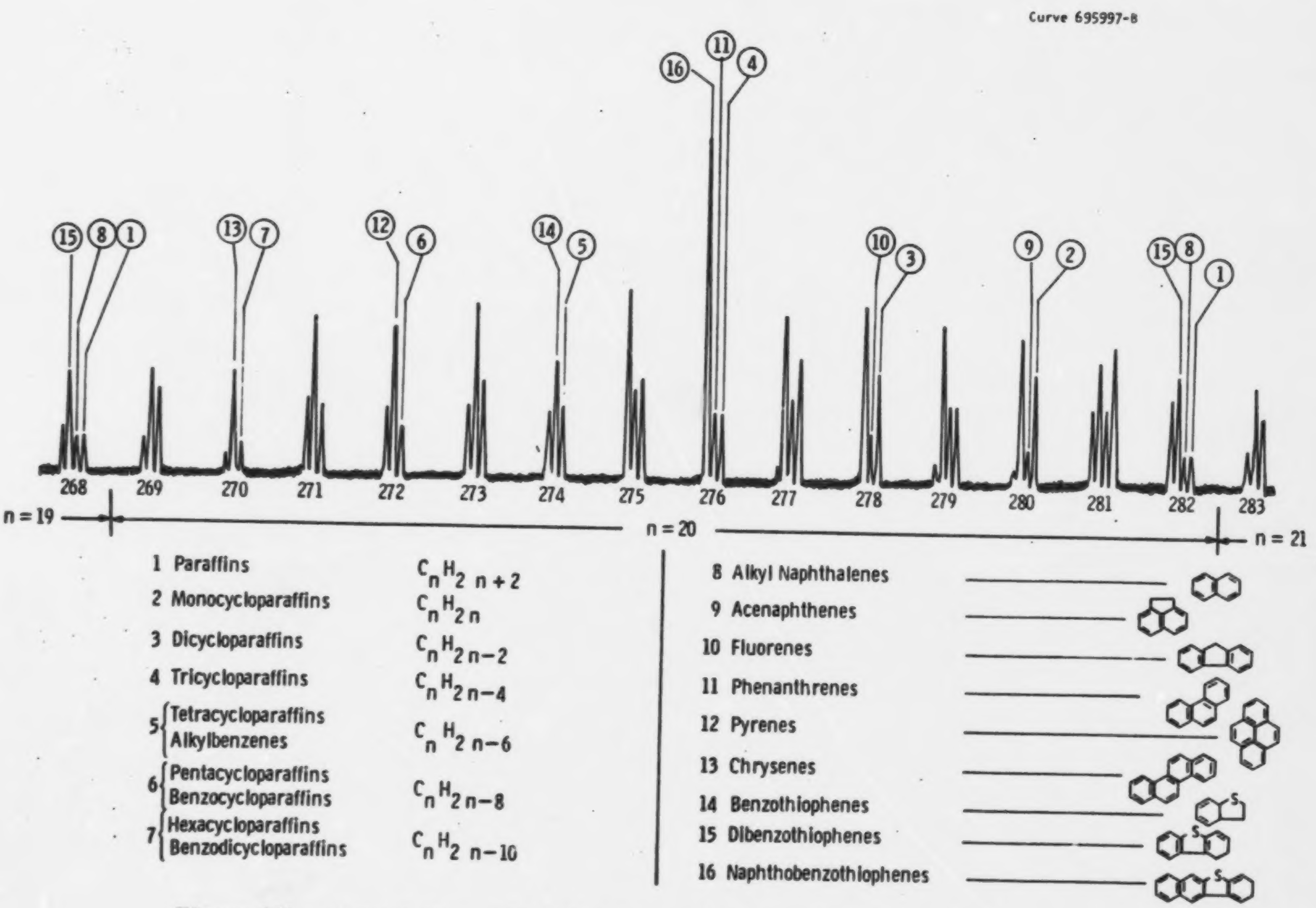


Figure 114. Segment From a High-Resolution Mass Spectrum of the 800°-950°F Fraction of an Arabian Crude

combined effect was strong. A 1971 review of carcinogens in oil by Catchpoole et.al.[53] discussed the difficulties in the measurement of the concentration of polycyclic aromatics. A book by Sergienko[54] discusses at great length the carcinogenic activity of polycyclic aromatic compounds and the effect of petroleum processing on their formation and presence. He points out that the presence of these materials is very rare in crude oil. However, the refining operations (Pyrolysis, distillation, catalytic cracking) favor the condensation of the mono aromatic rings into polycyclic ones. He also notes that the carcinogenic activity of condensed aromatic structures is also affected by the presence of hetero atoms in the nucleus. Sulfur-containing condensed cyclic structures are highly carcinogenic. The shift of the hetero atom in the condensed cyclic structure may also have a strong effect on its activity. Recent reviews on "Polynuclear Aromatic Carcinogens" and "Soots, Tars and Oils as Causes of Occupational Cancer" can be found in a 1976 ACS[55] monograph. Toxicity[56] of oils in Europe and Great Britain was reviewed at a 1974 workshop.

The toxicity of trace metals in oil is usually considered as an air pollution problem because of the use of residual oils as fuel. Table 59 is taken from an article by Vitez[57] and shows the concentrations and relative toxicities of metals in air. The largest toxicity factor is that for vanadium at 1800. Thus its relative ranking (order) is number one. Table 60 is taken from the same article by Vitez[57].

Table 59

TYPICAL TRACE ELEMENT CONCENTRATIONS IN RESIDUAL OIL AND RANKINGS IN ORDER OF THEIR POTENTIAL FOR HAVING HARMFUL EFFECTS AS EMISSIONS[57]

Element	Ppm Median	TLV mg/m³	Toxicity Factor	Toxicity Order
Sulfur	12,000	13	920	2
Silicon	300	—	—	—
Vanadium	180	0.1	1,800	1
Iron	150	10	15	10
Nickel	120	1	120	3
Calcium	120	5	24	8
Potassium	80	—	—	—
Aluminum	75	—	—	—
Magnesium	75	15	5	16
Sodium	35	2	17.5	9
Chlorine	12	3	4	18
Barium	4	0.5	8	13
Zinc	4	5	0.8	23
Phosphorous	4	0.1	40	4
Chromium	3	0.5	6	14
Cobalt	3	0.1	30	5
Manganese	2.5	5	0.5	24
Copper	2.5	0.1	25	6
Lead	2	0.2	10	11
Zirconium	2	5	0.4	25
Rubidium	2	—	—	—
Neodymium	1	—	—	—
Yttrium	1	0.5	2	20
Selenium	1	0.2	5	15
Titanium	0.4	—	—	—
Tin	0.3	0.1	3	19
Silver	0.2	0.01	20	7
Cerium	0.2	—	—	—
Thulium	0.2	—	—	—
Boron	0.3	15	0.02	33
Ruthenium	0.2	—	—	—
Bromine	0.28	0.7	0.4	26
Molybdenum	0.25	5	0.05	30
Cadmium	0.2	0.1	2	21
Platinum	0.02	0.002	10	12
Iodine	0.15	1	0.15	28
Strontium	0.1	—	—	—
Praseodymium	0.1	—	—	—
Tellurium	0.1	0.1	1	22
Germanium	0.1	—	—	—
Gadolinium	0.1	—	—	—
Hafnium	0.05	0.5	0.1	20
Tantalum	0.05	—	—	—
Terbium	0.05	—	—	—
Osmium	0.01	0.002	5	17
Arsenic	0.02	0.5	0.04	32
Antimony	0.02	0.5	0.04	31
Lanthanum	0.02	—	—	—
Mercury	0.01	0.05	0.2	27
Erbium	0.01	—	—	—
Scandium	0.01	—	—	—
Ytterbium	0.01	—	—	—
Cesium	0.005	—	—	—
Tungsten	0.005	1	0.005	35
Uranium	0.002	0.2	0.01	34
Dysprosium	0.002	—	—	—
Samarium	0.002	—	—	—
Lutetium	0.001	—	—	—
Holmium	0.001	—	—	—
Iridium	0.001	—	—	—
Palladium	0.001	—	—	—
Europium	0.0003	—	—	—
Gold	0.002	—	—	—
Rhenium	0.002	—	—	—
Indium	0.0002	0.1	0.002	36
Nitrogen	700	—	—	—

Table 60

TOXICITY RATINGS FOR 37 ELEMENTS IN AIR, OIL AND COAL[57]

Element	Toxicity Order			Mean Air Conc.	Toxicity Factor		
	In Air	In Oil	In Coal	ng/m³	Air	Oil	Coal
Vanadium	1	1	4	660	6,600	1,800	400
Lead	2	11	18	500	2,500	10	50
Sulfur	3	2	1	10,000	770	920	2,000
Sodium	4	9	6	1,530	765	17.5	400
Calcium	5	8	3	3,000	600	24	500
Iron	6	10	2	4,000	400	15	1,500
Cadmium	7	21	26	40	400	2	20
Bromine	8	26	24	160	230	0.4	20
Copper	9	6	9	220	220	25	250
Chlorine	10	18	5	620	207	4	400
Silver	11	7	11	1.2	120	20	200
Magnesium	12	16	12	1,160	73	5	167
Zinc	13	23	19	300	60	0.8	40
Chromium	14	14	17	26	52	6	50
Mercury	15	27	20	2	40	0.2	40
Nickel	16	3	7	26	26	120	250
Antimony	17	31	30	7	14	0.04	1
Selenium	18	15	23	2.4	12	5	25
Cobalt	19	5	15	1.2	12	30	100
Arsenic	20	32	25	4	8	0.04	20
Manganese	21	24	28	25	5	0.5	3
Iodine	22	28	29	4	4	0.15	2
Tungsten	23	34	34	0.6	0.6	0.005	0.3
Indium	24	35	35	0.05	0.5	0.002	0.1
Phosphorus	—	4	—	—		6	—
Barium	—	13	16	—		8	60
Zirconium	—	25	22	—		0.4	30
Yttrium	—	20	8	—		2	250
Tin	—	19	14	—		3	120
Boron	—	33	—	—		0.02	—
Molybdenum	—	30	32	—		0.05	0.4
Platinum	—	12	13	—		10	150
Tellurium	—	22	27	—		1	20
Hafnium	—	29	33	—		0.1	0.3
Osmium	—	17	21	—		5	40
Uranium	—	34	31	—		0.01	0.5
Beryllium	—	—	10	—		—	250

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